STUDY ON CARDIOPROTECTIVE EFFECT OF ENALAPRIL IN PATIENTS WITH BREAST CANCER ON DOXORUBICIN CHEMOTHERAPY.

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TAMILNADU.

Madurai

.09.2014

CERTIFICATE

This is to certify that the dissertation entitled **"STUDY ON CARDIOPROTECTIVE EFFECT OF ENALAPRIL IN PATIENTS WITH BREAST CANCER ON DOXORUBICIN CHEMOTHERAPY"** is a bonafide record of work done by **Dr.J.Arun kumar**, under the guidance and supervision of **Dr.S.Vijayalakshmi M.D.**, Professor, in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of his postgraduate study of M.D Pharmacology from 2012-2015.

Dr. R. PARAMESWARI. M.D.,
Director & Professor,
Institute of Pharmacology,
Madurai Medical College,
Madurai.

Captain. Dr.B. SANTHAKUMAR,
M.Sc (F.Sc), M.D (F.M),PGDMLE, DNB (F.M).,
Dean,
Madurai Medical College &
Govt. Rajaji Hospital, Madurai.

Madurai

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Dr. S. VIJAYALAKSHMI M.D.,
Professor,
Institute of Pharmacology,
Madurai Medical College,
Madurai.

DECLARATION

I, **Dr.J.Arun kumar** solemnly declare that the dissertation titled **"STUDY ON CARDIO PROTECTIVE EFFECT OF ENALAPRIL IN PATIENTS WITH BREAST CANCER ON DOXORUBICIN CHEMOTHERAPY"** has been prepared by me under the able guidance and supervision of **Dr. R. Parameswari M.D,** Director and Professor, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfillment of the regulation for the award of M.D Pharmacology degree examination of the Tamilnadu Dr. MGR Medical University, Chennai to be held in April 2015.

This work has not formed the basis for the award of any degree or diploma to me, previously from any other university to anyone.

Place: Madurai **Dr. J.ARUN KUMAR**

Date:

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ANNEXURES

STUDY ON CARDIOPROTECTIVE EFFECT OF ENALAPRIL IN PATIENTS WITH BREAST CANCER ON DOXORUBICIN CHEMOTHERAPY

AIMS AND OBJECTIVES:-

To determine the Cardioprotective effect of Angiotensin Converting Enzyme Inhibitor, Enalapril on Doxorubicin Induced Cardiotoxicity in breast cancer patients.

METHODOLOGY:-

The present study was carried out in the inpatients of Department Medical Oncology, Government Rajaji Hospital, Madurai after obtaining Institutional Ethical Committee Clearance. 60 female Breast cancer patients undergoing doxorubicin based chemotherapy were included for the study. Patients with Left ventricular ejection fraction (LVEF) >50% were taken in to the study. Patients were allocated into two groups 30 in each. All the 60 patients treated with FAC Chemotherapy regimen (5-Fluorouracil 500mg/m^2, Doxorubicin 50mg/m^2, Cyclophosphamide 500mg/m^2) once in 3 weeks for 6 cycles. Test group received Tab. Enalapril 5 mg / once daily at bed time started after the 6^{th} cycle of chemotherapy schedule and slowly titrated up to 10 mg once daily and continued for 6 months. Cardiac assessment was done by measuring Troponin I level at

baseline,24 hrs after first dose of chemotherapy and at the end of the chemotherapy schedule (6^{th} cycle). Cardiac function was also evaluated by serial measurement of Left ventricular ejection fraction (LVEF) and Fractional Shortening (FS) by echocardiogram at baseline, 3^{rd} cycle, 6th cycle, 6^{th} month and 9^{th} month of the study.

RESULTS:-

All the 60 patients were followed up to the end of the study. There was no drop out from the study. 23.3% of patients in both the groups showed persistent elevation of Troponin I level and those subjects were considered as High risk groups.

The mean LVEF at 9^{th} month in Enalapril treated and control groups were 61.90 ±2.34 & 54.57 ± 5.86 respectively. At the end of 9^{th} month the mean LVEF was maintained in Enalapril treated group than in control group from baseline line value which is statistically significant ($p < 0.001$).

The mean FS at 9^{th} month in Enalapril treated and control groups were 34.07 ±2.21 & 28.97 ± 3.47 respectively. When FS is compared between these two groups Enalapril treated group showed significant improvement in FS than in control group ($P < 0.001$).

Sub clinical toxicity defined as more than 10% reduction of LVEF from its baseline value during serial echocardiogram evalution. At the end of study 36.7% & 3.3% of patients showed subclinical cardiotoxicity in control and Enalapril group respectively. Cardiac events were significantly higher in control subjects than in Enalapril treated subjects.

CONCLUSION:-

Thus we conclude that the prognostic role of TnI as an early marker of cardiotoxicity to find out the high-risk patients and Prophylactic Enalapril administration have been showed to preserve the left ventricular function & improved cardiac outcome. Thus early treatment with Enalapril seems to prevent the development of late cardiotoxicity in patients undergone doxorubicin based chemotherapy.

KEY WORDS:-

Doxorubicin, Cardiotoxicity, Troponin I, Enalapril, Left ventricular ejection fraction, Fractional Shortening.

INTRODUCTION

Breast cancer is the second most common cause of cancer in females in India [1], contributing to major cause of morbidity & mortality. Breast cancer can be treated by multimodality approaches like surgery, radiotherapy, chemotherapy & hormonal therapy. The different types of chemotherapy for carcinoma breast vary according to the stage whether prior surgery has been done or not. It may be adjuvant, neoadjuvant and palliative chemotherapy [2].

The drugs commonly used to treat breast cancer include cyclophosphamide, methotrexate, doxorubicin, 5 flurouracil, pacletaxel, docetaxel, carboplatin, trastuzumumab. Most chemotherapeutic agents are reported to cause severe adverse reactions and some of which lead to organ damage [3]. But these agents cannot be avoided in the treatment of cancer though side effects cannot be abolished.

Major limitations to the clinical efficacy of chemotherapy have been toxicity to the normal tissues of the body and the development of drug resistance. In the past decade, better understandings of molecular biology and pathways/targets have led to target specific therapy. This has resulted in a paradigm shift in the management of many cancers.

Doxorubicin has been used as an efficacious antitumor antibiotic for many solid and haemopoietic malignancies. Doxorubicin (DOX), is an

anthracycline antitumor agent, plays vital role in the management of breast cancer. However, dose-dependent increased risk of heart failure and dilated cardiomyopathy has restricted its clinical use. Cardiotoxicity may compromise the efficacy of chemotherapy and affecting the quality of life & survival of the patients undergoing cancer chemotherapy. Risk factors [4] for doxorubicin induced cardiotoxicity are cumulative dose above 550 mg/m^2, more than 60yrs of age, dosing schedule, mediastinal radiotherapy, previous cardiac disease, hypertension, female sex and combined chemotherapy with known cardiotoxic agents like cyclophosphamide,trastuzumab etc.

Multiple mechanisms may contribute to the development of chemotherapy induced cardiotoxicity. However free radicals formation and oxidative stress to the heart appears to be an important cause of apoptosis and cardiomyocyte damage[5]. Doxorubicin induced Cardiotoxicity may develop during and delayed years after the treatment schedule with doxorubicin. Acute cardiotoxicity may manifests as tachyarrhythmia, pericarditis, myocarditis and even heart failure, can develop within weeks to months following treatment. Chronic cardiotoxicity may manifest as severe left ventricular dysfunction, dilated cardiomyopathy and chronic heart failure months to years after treatment which is not response to conventional treatment and become irreversible.

Hence patients undergoing anthracycline treatment need serial measurements of LVEF, Fractional shortening by echocardiography [6] prior to, during and after treatment to assess the left ventricular function and cardiotoxicity. Cardiac Troponin I is one of the marker for early myocardial insult has been used to monitor doxorubicin induced cardiotoxicity. An elevation in plasma troponin I level following cancer chemotherapy may be an important tool to predict the poor cardiological outcome in patients with breast cancer[7].

Adjustment in doxorubicin dose is the main approach to prevent the development of cardiac dysfunction. A certain number of patients still develop severe cardiac dysfunction at doses less than 550 mg/m^2. Iron chelating agent dexrazoxane and analogues of anthracycline like epirubicin, idarubicin has been used to protect patients with evidence of early cardiotoxicity at medium doses of doxorubicin. Few studies found that dexrazoxane eventhough reduce the cardiotoxicity, may also reduce the antitumor efficacy of anthracyclines [8].

Angiotensin Converting Enzyme Inhibitors (ACEI) like captopril,enalapril have been traditionally used to delay the deterioration of left ventricular function in many different clinical settings including doxorubicin induced cardiomyopathy [9]. Hence ACE inhibitors may be

useful in preventing doxorubicin induced cardiotoxicity by minimizing oxidative stress [10] and limiting left ventricular remodeling.

Many experimental animal model data's found & suggest that the Renin-Angiotensin System (RAS) plays a vital role in the formation and progression of doxorubicin-induced cardiotoxicity. Hence ACEIs like enalapril has been used to prevent & treat the anthracycline-induced cardiotoxicity. So patients, who are more prone to develop cardiotoxicity in future after exposure to doxorubicin, could have been prevented by prophylactic administration of ACEIs.

AIMS

&

OBJECTIVES

AIM AND OBJECTIVES

- To study the Cardioprotective effect of Enalapril on Doxorubicin based chemotherapy in breast cancer patients.
- To check whether Enalapril has got any protective effect on left ventricular function in doxorubicin induced cardiotoxicity.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

- Introduction to Breast Cancer
- Cancer Chemotherapy
- Doxorubicin Induced Cardiotoxicity
- Cardioprotectants
- ACE Inhibitors and Oxidative Stress
- Role of ACE Inhibitors in Doxorubicin Induced Cardiotoxicity

BREAST CANCER

Cancer is defined as a condition in which class of diseases with a group of cells display undifferentiated, uncontrolled growth, invasion via the blood, lymphatic and metastasis to the most of the organ of the body. The treatment of cancer involves surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapy.

According to WHO 2012, Global burden increases to 14 million new cases and 8 million cancer related deaths in 2012. Lung cancer (13.0%) followed by breast cancer (11.9%) are the commonly diagnosed cancers across the world. More than 50% of all cancers and death related to the cancers in 2012 occurred in developing countries like India and these proportions are expected to increase further by 2025 [12].Since the 2008 estimates, the incidence of breast cancer has been raised by more than 20% and mortality has been raised by 14%. The most commonly diagnosed cancer & the most common cause of cancer related death among women across the world is carcinoma breast[12] , which represents one in four of all cancers in women.

In India, carcinoma breast is the second most common malignancy among women next to cancer cervix. Since it presents as painless lump, patients neglect and come to hospital often late.

ETIOLOGY AND RISK FACTORS OF BREAST CANCER [13]

Etiology

Familial in 2-5% cases, BRCA1 & BRCA2 mutations in 50-70%,Li-Fraumen's syndrome, ataxia telangiectasia, Cowden's syndrome, p53 mutation, Hormone replacement therapy and oral contraceptive pills intake for more than 5 years.

Risk factors for breast cancer

Moderate risk

Florid hyperplasia, solid duct papilloma, Obesity, alcohol, Hormone Replacement Therapy, nulliparity, age > 35 years at first birth, early menarche and late menopause.

High risk

Age more than 60 years, breast cancer in one side, proliferative benign breast diseases like lobular carcinoma in situ and atypical ductal hyperplasia[14], H/O Ductal carcinoma in situ, mammographic dense breast.

Very high risk

Radiation exposure to chest, family H/O breast cancer in first degree relatives, family H/O breast and ovarian cancer,BRAC1 & BRCA2 mutation carrier or first degree relatives with this mutation[14].

BREAST CANCER CLASSIFICATION [15]

I. **Non-invasive epithelial cancer**

- LCIS - Lobular Carcinoma In Situ
- DCIS - Ductal Carcinoma In Situ – solid, papillary & comedo

II. **Invasive epithelial cancer**

- Invasive lobular – 10%
- Invasive ductal – 70%
- Medullary carcinoma – 5%
- Tubular, colloid, cribriform – each 2%
- Invasive papillary, metaplastic, adenoid cystic – each 1%

III. **Mixed connective tissue and epithelial**

- Phylloides, angiosarcoma

CLINICAL PRESENTATION[13,14]

Most women with carcinoma breast will present with lump in the breast which is hard, painless and may be associated with indrawing of nipple, nipple discharge, ulceration and fungation, axillary / supraclavicular node enlargement, chest pain, haemoptysis, bone pain, pathological fractures, pleural effusion and ascites.

INVESTIGATIONS [13]

Mammography:- Women between 40-49 years-studies should be done every 12 to 24 months. Annual mammogram to be done for those >50 years and women younger than 50 years of age who are in high-risk group.

- Ultrasound:- Useful in young female with dense breast in whom diagnosis is difficult to interpret. Used to localise impalpable areas of breast pathology.
- CT scan :- aid in clinical staging of malignant processes.
- MRI :- Best imaging modality for the breast of women with implants.
- FNAC:- done in palpable mass, mass on mammogram.
- Biopsy:- Excision & Incision
- Chest X-ray:- Lung metastasis, pleural effusion
- Bone scan:- Identify occult osseous metastases
- Liver enzymes:- SGOT,SGPT,ALP – For liver metastasis.
- Hormone receptor status:- ER,PR status- to guide adjuvant therapy.
- HER-2/neu receptor:- to predict prognosis and has better response to adriamycin therapy.
- Triple assessment:-Combination of physical examination, mammography and FNAC will produce a diagnostic accuracy approaching 100%.

Table – 1 BREAST CANCER - TNM STAGING SYSTEM [15]

T_X	Primary cancer could not be assessed
T_0	Absence of evidence of primary cancer
T_{is}	Carcinoma in situ
$T_{is\ (DCIS)}$	Ductal carcinoma insitu
$T_{is\ (LCIS)}$	Lobular carcinoma insitu
$T_{is(Paget's)}$	Paget's disease of the nipple without tumor
T_1	Tumor size 2 cm in greater dimension
T_{1mic}	Microinvasion 0.1 cm or less in greater dimension
T_{1a}	Tumor size >0.1 cm but not >0.5 cm in greater dimension
T_{1b}	Tumor size >0.5 cm but not >1 cm in greater dimension
T_{1c}	Tumor size >1 cm but not >2 cm in greater dimension
T_2	Tumor size >2 cm but not >5 cm in greater dimension
T_3	Tumor size >5 cm in greatest dimension
T_4	Any tumor size with extension to Skin or chest wall
T_{4a}	Extents to chest wall, pectoralis muscle not involved
T_{4b}	Edema (peau d'orange), or skin ulceration
T_{4c}	Both T4a and T4b
T_{4d}	Inflammatory carcinoma

Table – 2 Regional lymph nodes—Clinical (N)

N_0	No regional lymph node metastasis
N_1	Axillary nodes- ipsilateral,mobile,discrete
N_{2a}	Axillary nodes- ipsilateral fixed
N_{2b}	Metastasis to I/L internal mammary nodes only
N_{3a}	Metastasis to I/L infraclavicular & Axillary nodes
N_{3b}	Metastasis to I/L internal mammary & Axillary nodes
N_{3c}	Metastasis to I/L supraclavicular lymph nodes

Table – 3 Distant metastasis (M)

M_X	Couldnot be assess the distant metastasis
M_0	Absence of distant metastasis
M_1	Presence of distant metastasis

Table – 4 TNM Stage Groupings[15]

Stage 0	$TisN_0M_0$				
Stage I	$T_1N_0M_0$				
Stage IIa	$T_0N_1M_0$	$T_1N_1M_0$	$T_2N_0M_0$		
Stage IIb	$T_2N_1M_0$	$T_3N_0M_0$			
Stage IIIa	$T_0N_2M_0$	$T_1N_2M_0$	$T_2N_2M_0$	$T_3N_1M_0$	$T_3N_2M_0$
Stage IIIb	$T_4N_0M_0$	$T_4N_1M_0$	$T_4N_2M_0$		
Stage IIIc	AnyT N_3M0				
Stage IV	AnyT,Any N,M_1				

Treatment options in Carcinoma of Breast [14,16]

Pimary - Surgery

- Lumpectomy - Wide local excision
- Simple/total mastectomy
- MRM - Modified Radical Mastectomy
- Radical mastectomy
- Axillary lymph node dissection

Adjuvant

A. Radiotherapy

- Post operative radiotherapy & Palliative radiotherapy

B. Systemic therapy

- **Hormonal therapy –** Tomoxifen, Raloxifene, Letrozole.

- **Chemotherapy**

In 1960s first trails of combination chemotherapy were initiated for breast cancer management. First adjuvant chemotherapy was administered to women with positive nodes; later in 1980s the use was extended to node negative women as well.

Indications for chemotherapy [13,15]

The proportional reduction in recurrences and mortality in both node positive and negative patients are similar, but given the better prognosis of node negative patients especially those node negative with small tumors(<1 cm). Younger females have proportionally greater reduction in both mortality and recurrence than older females with carcinoma breast. Combination chemotherapy has been more effective than single agent therapy.

DOSAGE AND SCHEDULE

Choosing the regimen

There is no single regimen that has emerged as the treatment of choice. Several trials have demonstrated that a 10% - 20% higher response rate has been observed with Doxorubicin/Epirubicin containing regimen with rise in median survival from 12-18 months and rise in median time of treatment failure from 4 to 6 months.

REGIMENS [14,16]

FAC:-5 Fluorouracil: 500mg/m^2, Adriamycin: 50mg/m^2, Cyclophosphamide: 500mg/m^2, On day one & every 3 weeks for 6 cycles.

AC:-Adriamycin: 60mg/m^2, Cyclophosphamide: 600 mg/m^2 - 4 cycles are given, once in every 3 weeks.

CMF:-Cyclophosphamide: 750 mg/m^2**,** Methotrexate: 50 mg/m^2,5-FU: 600 mg/m^2**-** Given once in every 3 weeks, for 6 cycles.

FEC:-5 FU 500mg/m^2,Epirubicin 50mg/m^2,Cyclophosphamide 500mg/m^2**-** On day one & every 3 weeks for 6 cycles.

TIMING OF TREATMENT [15,16]

Adjuvant therapy:- (ACT)

Chemotherapy is given after a surgery for cure with an aim to prevent local and systemic relapse by eradicating micrometastasis and to improve outcome in cancers like breast, ovary, colon etc.

Neo-adjuvant therapy (NACT)

Tumor down staging with NACT is used to convert inoperable tumor into operable one and to allow breast conservation surgery. Traditionally, neo adjuvant chemotherapy (NACT) has been used in locally advanced breast cancers that are inoperable.

Palliative therapy

Depending on the receptor status, distant sites and those experiencing distant relapse after adjuvant treatment are treated by palliative measures either by endocrine manipulation or chemotherapy. Patients who are candidates for chemotherapy are those who fail hormonal therapy or presence of visceral metastasis.

CANCER CHEMOTHERAPY

Chemotherapy, which includes newly developed targeted treatments, is the principle tool to treat most cancers. The development of effective combination chemotherapy for Hodgkin's lymphoma, childhood leukemia and lymphomas in the 1960s provided curative therapeutic strategies for patients with advanced malignancies of all types.

Historical perspective

Paul Ehrlich coined the term chemotherapy. Alkylating agents represent the first class of chemotherapeutic drugs to be used in the clinical setting. First clinical use of nitrogen mustard in a patient with non–Hodgkin's lymphoma was in 1942.

Clinical application of chemotherapy [17]

Chemotherapy is used in four main clinical settings:-

(a) Primary induction treatment for advanced cancers for which there are no other effective treatment. (b) As the primary or neoadjuvant treatment for patients with localized disease for which local forms of therapy, such as surgery, radiation, or both, are ineffective by themselves. (c) Adjuvant treatment for early-stage disease following local modes of treatment like radiotherapy, surgery or both. (d) Directly instilled into sites of specific regions of the body directly affected by the cancer.

Primary Chemotherapy:

Cancers for which chemotherapy is a primary treatment modality in cancers like Acute leukemia, Non-Hodgkin lymphoma, Myeloma, Hodgkin lymphoma, Germ cell cancer, Lymphoma, Ovarian cancer, Small cell lung cancer, Wilms tumor and Embryonal rhabdomyosarcoma.

Neoadjuvant Chemotherapy:

Cancers for which neoadjuvant chemotherapy is indicated for locally advanced diseases like Non–small cell lung cancer, Head and neck cancer, Bladder cancer, Ovarian cancer, Breast cancer.

Adjuvant Chemotherapy: Cancers for which adjuvant therapy is indicated after surgery in Pancreatic cancer, Melanoma, Breast cancer, Non–small cell

lung cancer, Osteogenic sarcoma, Colorectal cancer, Gastric cancer and Anaplastic astrocytoma.

Principles of cancer cell kinetics [2,17]

The antineoplastic agents follow the logarithmic cell-kill kinetics to exert their cytotoxic effects. Constant fraction of cells not numbers are killed by these drugs. If a anticancer agent leads to a 4 log kill of neoplastic cells and reduces the tumor burden from 10^{12} to 10^{8} cells, the same dose is used at a tumor burden of 10^{7} cells reduces the tumor mass to 10^{3}. Cell kill is therefore proportional, regardless of tumor burden.

CLASSIFICATION OF ANTI NEOPLASTIC AGENTS [18]

The compounds used in the chemotherapy of neoplastic disease are quite differed in molecular structure and mode of action, including antimetabolites, purine and pyrimidine; alkylating agents; products of natural source; hormones and hormonal antagonists; and a variety of agents directed at specific molecular targets.

Table – 5 ALKYLATING AGENTS

Type of agent	**Individual Drugs**
Nitrogen mustards	Chlorambucil, Mechlorethamine, Melphalan, Ifosfamide, Cyclophosphamide.
Methylhydrazine derivatives	Procarbazine
Triazenes	Temozolomide, Dacarbazine
Nitrosoureas	Streptozocin, Carmustine, Bendamustine
Alkyl sulfonate	Busulfan
Platinum complexes	Cisplatin, oxaliplatin, carboplatin.

Table – 6 ANTIMETABOLITES

Type of agent	**Individual Drugs**
Folate analogs	Pemetrexed, Methotrexate
Purine analogs	Pentostatin, Fludarabine, 6-Mercaptopurine , Clofarabine, Nelarabine
Pyrimydine analogs	Gemcitabine, capecitabine, 5-fluorouracil, Cytarabine, 5-aza-cytidine

Table - 7 NATURAL PRODUCTS

Type of agent	**Individual Drugs**
Vinca alkaloids	Vinblastine, Vinorelbine, Vincristine
Antibiotics	Doxorubicin, Daunorubicin, Dactinomycin
Epipodophyllotoxins	Teniposide, Etoposide
Camptothecins	irinotecan ,Topotecan
Taxanes	Paclitaxel, docetaxel
Echinocandins	Yondelis
Anthracenediones	Bleomycin, Mitoxantrone, Mitomycin C
Enzymes	L-Asparaginase

Table - 8 HORMONES AND ANTAGONISTS

Type of agent	**Individual Drugs**
Adrenocortical	Mitotane
Adrenocortico-steroids	Prednisone
Progestins	Medroxyprogesterone acetate, Hydroxyprogesterone caproate, Megestrol acetate
Estrogens	Ethinyl estradiol , Diethylstilbestrol
Anti-estrogens	Toremifene , Tamoxifen,
Aromatase inhibitors	Anastrozole, Letrozole, Exemestane
Androgens	Fluoxymesterone, Testosterone propionate
Anti-androgen	Casodex , Flutamide
GnRH analog	Leuprolide

Table – 9 MISCELLANEOUS AGENTS

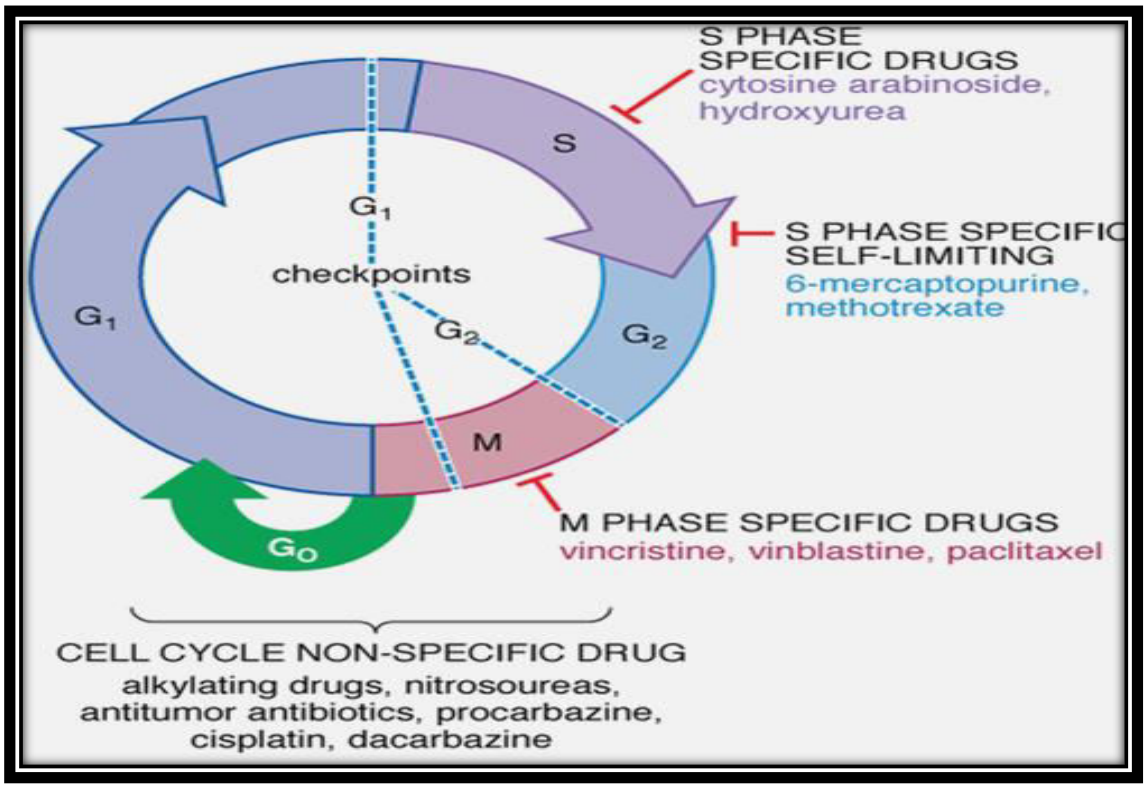
Type of agent	**Individual Drugs**
Substituted urea	Hydroxyurea
Differentiating agents	Tretinoin, Arsenic trioxide, vorinostat
Tyrosine kinase inhibitors	Gefitinib, Imatinib, Dasatinib, Nilotinib, Erlotinib Sorafenib, Sunitinib, Lapatinib
Proteasome inhibitor	Bortezomib
Biological response modifiers	Interleukin-2, Interferon-α
Immunomodulators	Thalidomide, Lenalidomide
Monoclonal antibodies	Temsirolimus, everolimus

THE CELL CYCLE [19]

Many cytotoxic agents act by damaging DNA. Their toxicity is greatest during the S, or DNA synthetic phase of the cell cycle. Others, vinca alkaloids and taxanes, block the formation of a functional mitotic spindle in the M phase. These agents are most effective on cells entering mitosis, the most vulnerable phase of the cell cycle. All cells display a similar pattern of cell cycle progression.

- Phase that precedes DNA synthesis (G_1)
- DNA synthetic phase (S)
- An interval which is followed by the termination of DNA synthesis (G_2)
- The mitotic phase (M) in which the cell, containing a double complement of DNA, divides into two daughter G_1 cells a probability of moving into a quiescent state (G_0) and failing to move forward for long periods of time.

Figure – 1 Cell cycle[18]



CELL CYCLE SPECIFICITY OF ANTINEOPLASTIC AGENTS [20]

Slowly growing tumors with a small growth fraction (e.g., carcinomas of the colon or non–small cell lung cancer) are less responsive to cycle-specific drugs. More effective are agents that inflict high levels of DNA damage (e.g., alkylating agents) or those that remain at high concentrations inside the cell for extended periods of time (e.g., fluoropyrimidines).

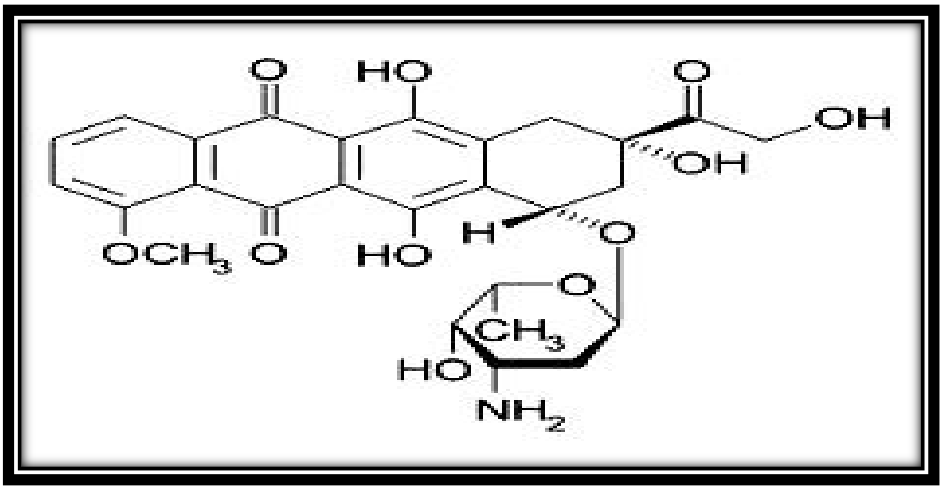
DOXORUBICIN

Doxorubicin otherwise known as Adriamycin was the first anthracycline isolated from Streptomyces peucetius [21] in the year 1963 by both Italian and French group of scientists. Italian group isolated natural product doxorubicin from Streptomyces peucetius var. caesius. The French group produced semi synthetic derivatives. Epirubicin and Idarubicin are analogs of daunorubicin and doxorubicin respectively, only slightly differ in their chemical structures. Doxorubicin exerts broad-spectrum activity against solid human cancers. These drugs have the ability to produce free radicals and cause an irreversible, unusual cardiomyopathy which is related to exposure of the total dose of the drug.

CHEMISTRY

The anthracyclines have a tetra cyclic ring structure attached to daunosamine which is an unusual sugar. These drugs having quinone and hydroxyquinone moieties on neighbor rings which permit the loss and gain of electrons. Their chemical structures differ from daunorubicin by only a single hydroxyl group on Carbon -14.

Figure – 2 Chemical Structure of Doxorubicin[21]



Absorption, fate, and excretion[20]

Doxorubicin is very poorly absorbed and is therefore administered parenterally. It is relatively rapidly distributed throughout the body including breast milk and bound to plasma proteins moderately. There is no evidence that it crosses the placenta but it may cause harm to the fetus. Doxorubicin is cleared by complex hepatic metabolism and excrete via bile. The plasma disappearance curve is triphasic, with the first phase $t_{1/2}$ 10-20 mts due to distribution, the second phase 1.5 - 10 hrs largely to metabolism and last phase due to release from binding sites such as DNA and cardiolipin with a terminal $t_{1/2}$ of 24-48 hrs.

Doxorubicin is converted into an alcohol intermediate [22] which plays a different role in its therapeutic activity. The drug rapidly enters the lungs, heart, liver, spleen and kidneys. It does not cross the blood-brain barrier. Doxorubicin is eliminated by its metabolic conversion into aglycones and few inactive products. Idarubicin is mainly metabolized into idarubicinol that accumulates in plasma and which is responsible of its activity. In the presence of hepatic failure the clearance of anthracyclines and their active alcohol metabolites are delayed. 50% of initial dose reduction should be considered in patients if serum bilirubin level is elevated.

FDA approved indications of doxorubicin[18]

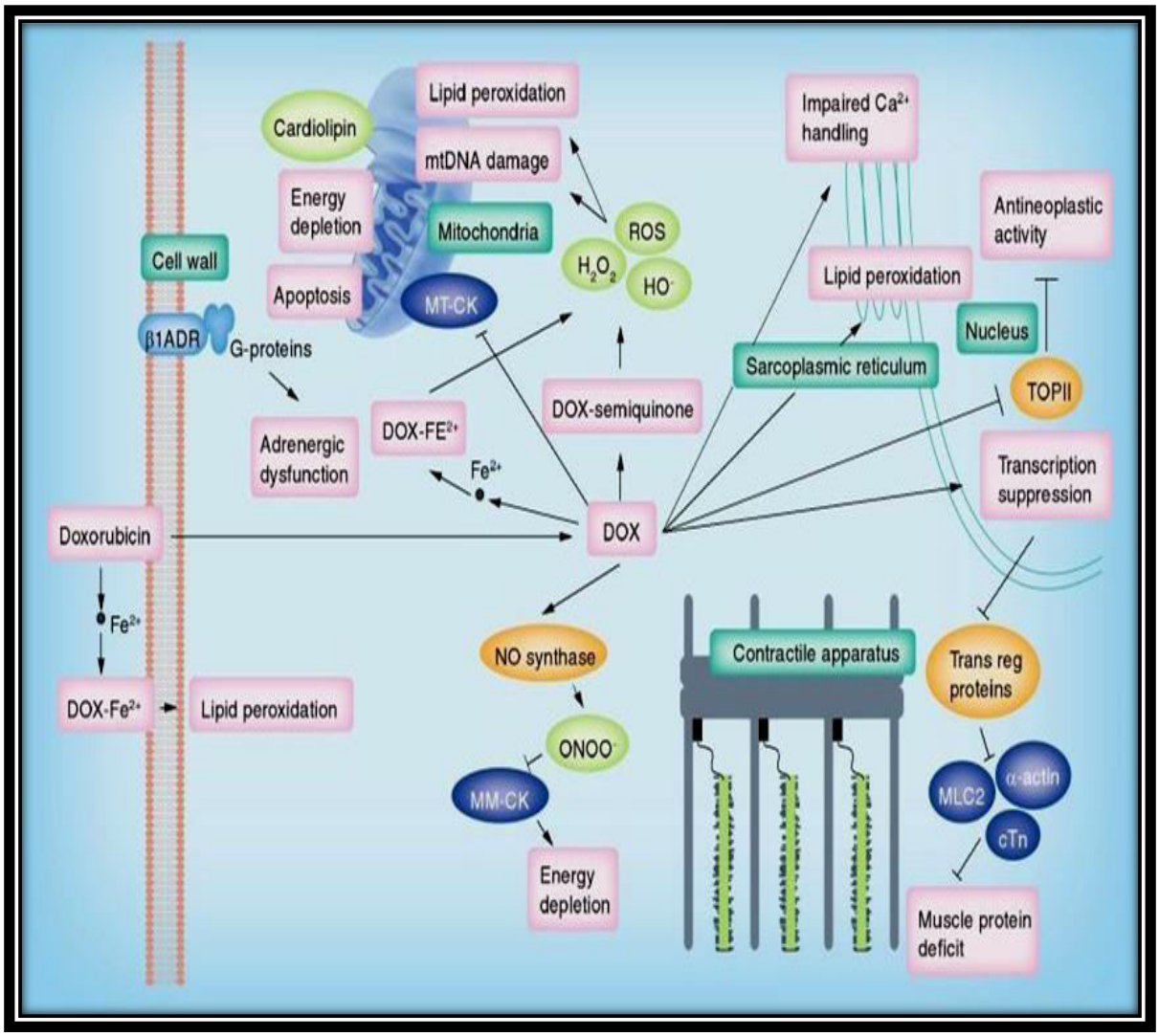
Hodgkin' s lymphoma, ALL , AML, CLL, Non Hodgkin' s lymphoma, Multiple myeloma, Mycosis fungoides, Mantle cell lymphoma, Kaposi sarcoma, Breast cancer (adjuvant and advanced disease), Advanced Prostate cancer, Gastric cancer, Ewing's sarcoma, Thyroid cancer. In combination with cyclophosphamide, it is an important component of different regimens used as adjuvant chemotherapy and in metastatic carcinoma of the breast [19].

Mechanism of action [18,20]

Anthracyclines are directly affecting the transcription and replication of the neoplastic cells by intercalating with DNA. Their important action is mediated by their ability to form a tripartite complex with DNA and topoisomerase II. Topoisomerase II is an ATP-dependent enzyme that binds to DNA. It causes double-strand nicks at the 3'-phosphate backbone and allowing strand passage and uncoiling of super-coiled DNA. Then topoisomerase II religates the DNA strands. This enzymatic function is important for DNA replication and repair. The tripartite complex formation with anthracyclines or with etoposide inhibits the re-ligation of the broken DNA strands, which leads to apoptosis. Any defect in DNA double-strand break repair sensitizes neoplastic cells to damage by these drugs. But over expression of transcription-linked DNA repair may lead to drug resistance.

Anthracyclines generates free radicals in solution and in both normal and malignant tissues because of their quinone moieties. Anthracyclines can form semiquinone radical intermediates [22] that can react with O_2 to produce superoxide anion radicals which is responsible of their antitumor activity and also its cardiotoxicity.

Figure-3 Mechanism of action of doxorubicin



These can produce both hydroxyl radicals and hydrogen peroxide which disrupt DNA and oxidize DNA bases. Iron interacts with doxorubicin and stimulates the free radicals production significantly. Catalase and superoxide dismutase are defensive enzymes that protect cardiac cells against the anthracyclines toxicity. These defense mechanisms may be augmented by exogenous administration of antioxidants such as alpha tocopherol. Dexrazoxane an iron chelating agent which protects against

cardiotoxicity. Anthracyclines exposure to the myocardial cells to leads to apoptosis. This process is mediated by p53 – a DNA-damage sensor and proteases, activated caspases, ceramide, and the Fas receptor-ligand system also have been implicated.

Dosage and administration [22]

Doxorubicin is usually given as a single intravenous infusion dose of 60-75 mg/m^2 slowly over 4 to 5 minutes that is repeated after 3 weeks. Since it is a vesicant, care must be taken to avoid extravasations, tissue necrosis. Drug can be given as a continuous infusion over 2-4 days through central venous access line. Dose reduction is needed in patients with liver failure. Reduce 50% of dose if serum bilirubin between 1.2 – 3.0 mg/dl [22]. Infuse only 25% of dose if sr.bilirubin level greater than 3 mg/dl. Weekly therapy may be more cytotoxic to cancer cells than comparable doses of monthly bolus schedules[36].

Drug interactions[21]

- Doxorubicin is a potent radiosensitizing agent and radiation recall reactions are potentially dangerous.
- Radiation may increase the risk of cardio toxicity of doxorubicin.
- Interferon potentiates doxorubicin efficacy in follicular lymphoma.

- Cyclosporine increases the toxicity of doxorubicin by inhibiting P-glycoprotein
- Vitamin D_3 enhances the doxorubicin induced oxidative damage in breast cancer cells
- Pacletaxel modifies the pharmacokinetic profile of doxorubicin.

CLINICAL TOXICITIES [18,22,23]

- Myelosuppression is the most common toxicity
- Stomatitis, mucositis, occurs in nearly 10% of patients.
- Total or near total alopecia [21] occurs in nearly every patient.
- Extravasations
- Severe nausea and vomiting are common
- Anorexia and diarrhoea may occur in less than 10% of patients.
- Facial flushing, conjunctivitis, and lacrimation.
- **Cardiotoxicity:-**[18,22] Cardiac toxicity is a rare unusual but peculiar adverse effect observed with these agents. It leads to tachycardia, arrhythmias, dyspnoea, hypotension, pericardial effusion, and congestive heart failure which is poorly responsive to digitalis.

CYCLOPHOSPHAMIDE

Cyclophosphamide is an alkylating agent and chemical derivative of mechlorethamine which was first synthesized in Germany in 1958 [26].It is used as a single agent to treat burkitt´s lymphoma. As combination chemotherapy [17] in carcinoma breast, ovary, lung and multiple myeloma. It is an immunosuppressant – used in nephritic syndrome, psoriasis.

Metabolism

- Causes Microsomal hydroxylation
- It is hydrolysis to Phosphoramide mustard (active) and acrolein.
- Excretion as inactive oxidation products.

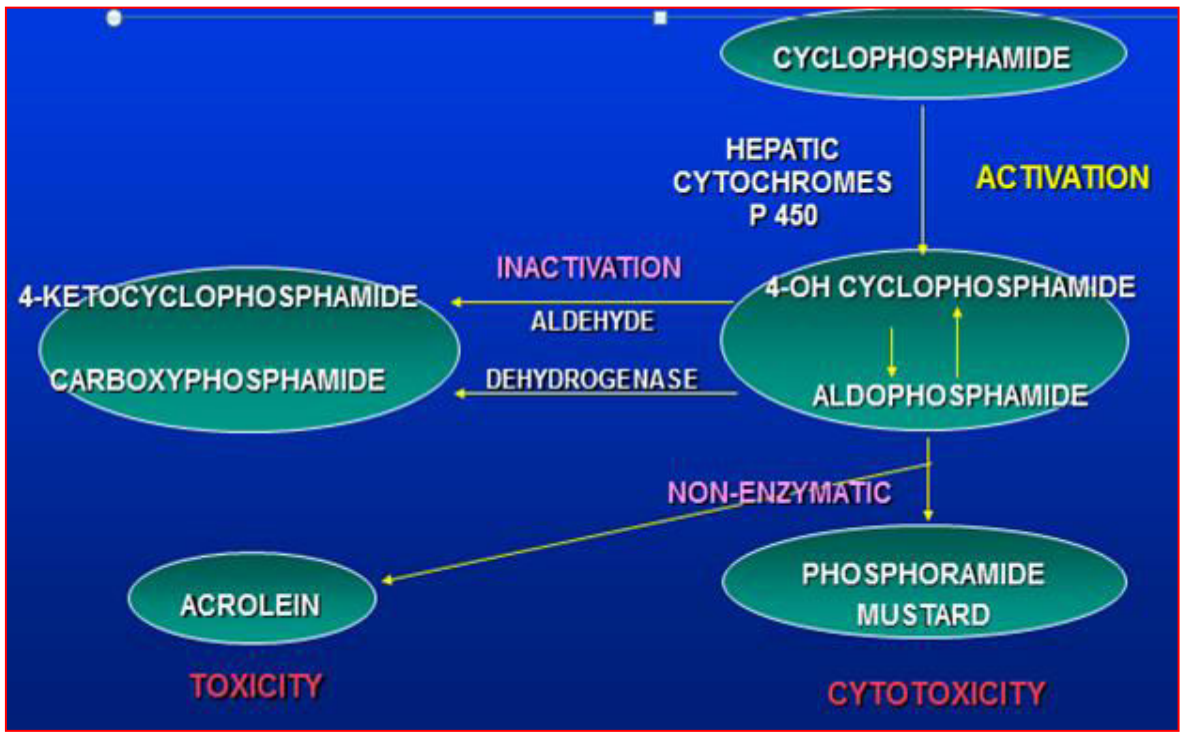
Mechanism of action

- Produce DNA alkylation via the formation of reactive intermediates that attack nucleophilic sites.
- Cell cycle non specific [20]

Dose schedule

- Intravenous – 400 to 2000 mg/m^2
- Oral – 100 mg/m^2 or 1 to 25 mg/kg/day in divided doses.

Figure – 4 Metabolism of Cyclophosphamide[18]



PHARMACOKINETICS

Bioavailability**:-** Oral >75 % ; Protein bound > 60 %

Primary elimination t ½:-Parent drug:- 3 – 10 hrs, Aldophosphamide:- 1.6 hrs, Phosphoramide mustard:- 8.7 hrs

Toxicity [18,19]

Myelosuppression is the major dose limiting toxicity of cyclophosphamide. It causes severe neutopenia than thrombocytopenia. Leucopenia develops after 8 – 14 days of therapy with recovery 1 or 2 weeks later. Other common side effects are alopecia, pigmented finger nails,

nausea, vomiting, pulmonary fibrosis, transient myopia, cataract, Haemorrhagic cystitis and SIADH.

Teratogenesis – category D in FDA

Cardiotoxicity [22,29,]

Dose limiting cardiac toxicity occurs when using 7 fold increased normal dose, ≥180 mg/kg for 4 or more days or > 1.55 g/m^2/day, mainly with transplantation doses. Mechanisms underlying the toxicity are believed to be injury of both endothelial cells and myocytes, and a picture of hemorrhagic myocardial necrosis can emerge. It causes low grade delayed cardiotoxicity which is not related with cumulative dose toxicity, more with patients older than 50 yrs of age. Maximum tolerated dose - 7000 mg/m^2

Precautions- Use MESNA (2- Mercaptoethane sulfonate) with high dose therapy.

Drug interactions[20]

- Increased cytotoxicity with radiation sensitizers and glutathione depletion.
- Inhibit pseudo cholinesterase – risk of apnoea with succinyl choline.
- Risk of cardiomyopathy when combine with anthracyclines.
- Cimitidine enhances myelosuppression of cyclophosphamide.

5 – FLUOROURACIL (5 – FU)

5-Fluorouracil is an antimetabolite and structural analogue of DNA precursor of thymine [22]. It is developed in 1957 by Heidelberger and Ansfield. It is used to treat carcinoma breast, gastrointestinal tract and many cancers.

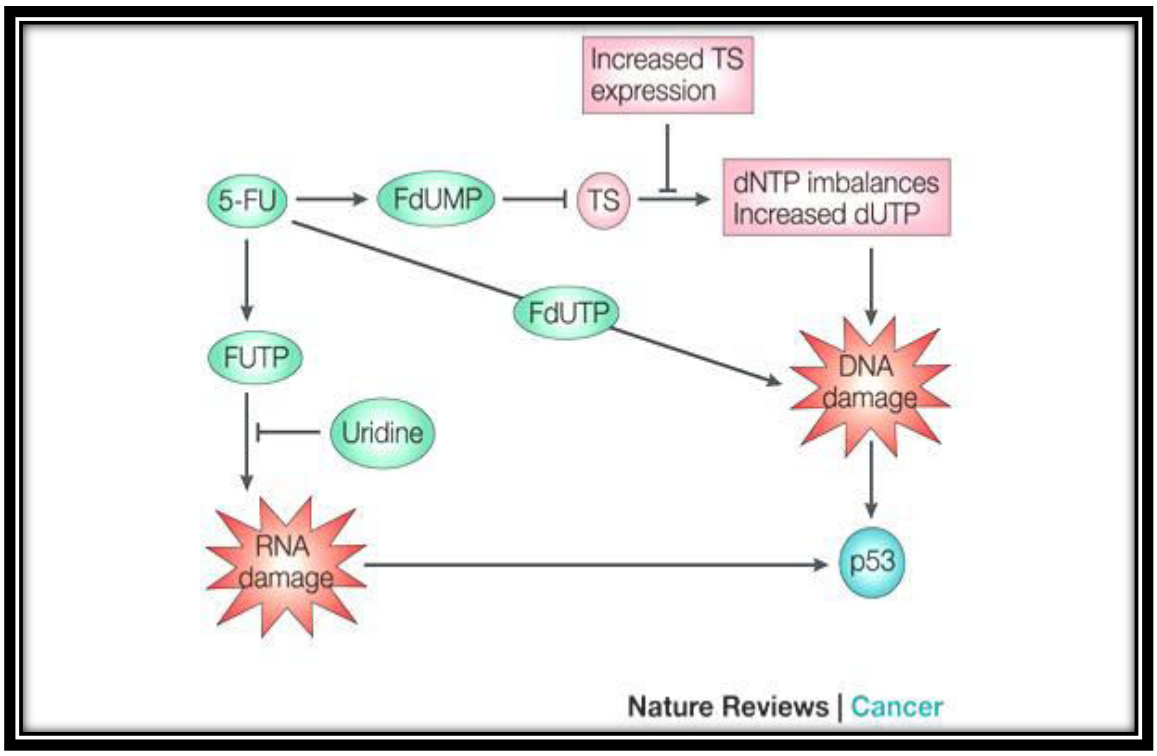
Metabolism

5-FU is a prodrug that enters cells and phosphorylated to series of metabolites. It is converted enzymatically to active nucleotide forms intracellularly. DPD (Dihydropyrimidine dehydrogenase) catalyzes the initial, rate limiting step in 5 FU catabolism.

Mechanism of action [18,22]

Fluorouridine triphosphate incorporated into RNA which interferes with RNA synthesis and its function. Thymidylate synthase inhibition is mediated by fluorodeoxyuridylate (FdUMP) which leads to thymidine 5´ monophosphate and thymidine 5´ triphosphate depletion. It also causes accumulation of deoxyuridine monophosphate and deoxyuridine triphosphate. Incorporation of fluorodeoxyuridine triphosphate and deoxyuridine triphosphate into DNA may affect DNA stability. Genotoxic stress triggers programmed cell death pathways.

Figure – 5 Mechanism of action of 5- Fluorouracil



Pharmacokinetics

- Half life is 8 – 14 mts after iv bolus infusion.
- Nonlinear pharmacokinetics due to saturable catabolism
- Total body clearance decreases with increasing doses.

Elimination

- 90% eliminated by metabolism; less than 10% excreted as unchanged in urine. 5 FU and its catabolites undergo biliary excretion.

Drug interactions

- Chronic administration of cimitidine –decrease 5-FU clearance.

- Dipyridamole increases 5 –FU clearance .
- Sequential Methotrexate (Mtx) use → Mtx increases 5- FU toxicity and increases fluorouridine triphosphate (FUTP) incorporation into RNA; may antagonize DNA directed toxicity of 5 –FU.
- Inhibitors of de novo pyrimydine synthesis increase 5 – FU anabolism to the ribonucleotide level and 5 FU – RNA incorporation.

Toxicity

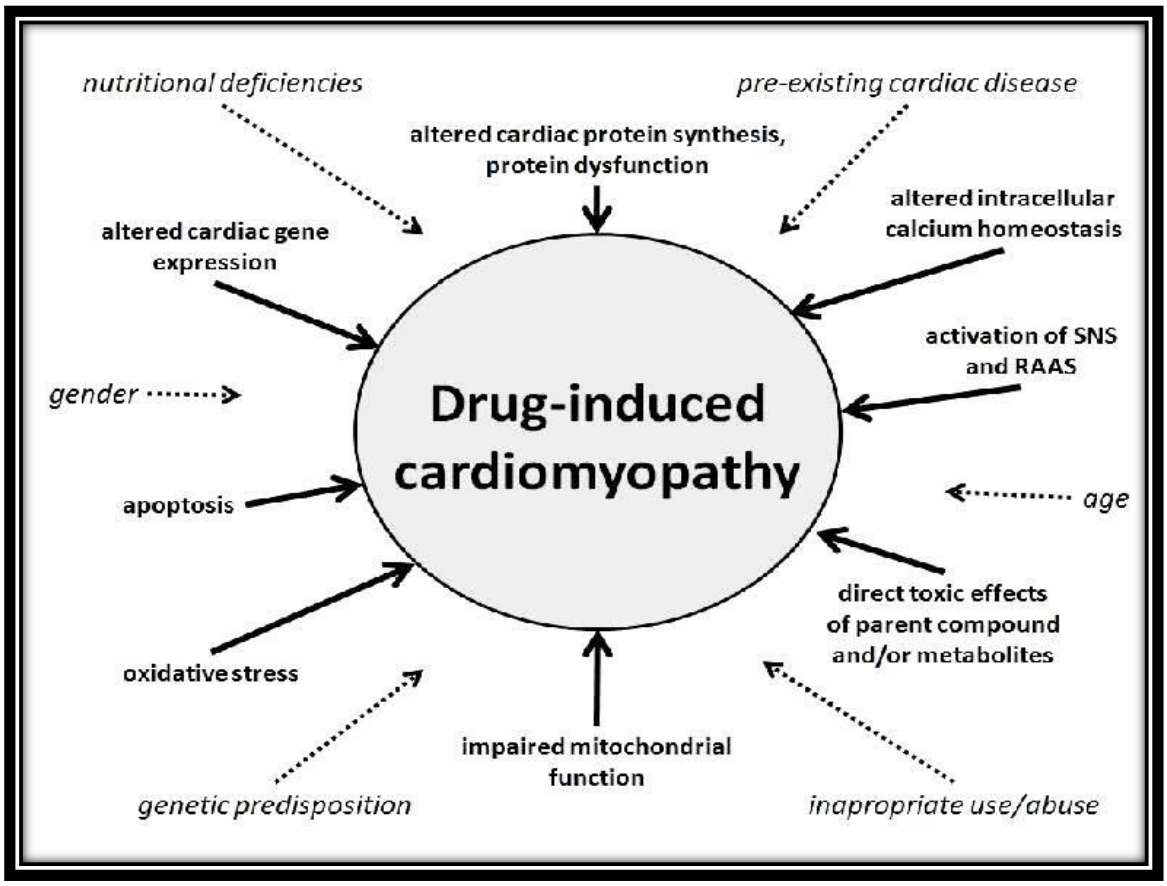
- Gastrointestinal epithelial ulceration, Myelosuppression, skin rashes
- Neurotoxicity – cerebellar ataxia, cognition dysfunction
- **Cardiac toxicity**
  - It causes coronary spasm [22] & myocardial ischemia (1.6%) [29].
  - More common with high continuous infusion than iv bolus administration. Fluorouracil can cause acute ischemic syndromes ranging from angina to MI and this can occur in patients without CAD (approximately 1% of patients), although it is more common in patients with pre-existing disease (4% to 5%).
  - Vasospasm is believed to be the mechanism triggering ischemia, although thromboembolic events are also increased.

CHEMOTHERAPY INDUCED CARDIOTOXICITY [24]

There are 2 types of drug induced cardiac toxicities commonly observed. Type I cardiotoxicity is more serious and causes permanent damage to the myocardium and type II which is usually reversible.

Type I cardiotoxicity found in Anthracyclines, type II cardiotoxicity found in traztuzumab [23]. Anthracyclines induced cardiotoxicity is a cumulative dose dependent toxicity which is characterized by each administration constitutes additive or sequential damage to the heart.

Figure – 6 Chemotherapy induced cardiotoxicity[24]



ANTHRACYCLINE INDUCED CARDIO TOXICITY [23 - 30]

- Acute and sub acute cardiotoxicity
- Delayed onset- irreversible dilated cardiomyopathy

ACUTE TOXICITY [25]

Acute toxicity manifest during or soon after administration of the drug. It is generally minor and reversible and independent of anthracycline dose. Manifestations are asymptomatic ECG changes, Myocarditis, Pericarditis, transient heart failure may develop. Incidence - approximately 11 %

ECG changes [27,29] may be manifests are ST changes, Low voltage QRS complex, Poor R wave progression, Prolongation of QT interval, Atrial / ventricular ectopics, T wave inversion, supra ventricular tachycardia, ventricular arrhythmia. Conduction block is more common in paediatric age group.

An acute reversible reduction in ejection fraction is observed in some patients in the 24 hours after a single dose, and plasma troponin I, a cardiac enzyme released with myocardial damage, may increase in a minority of patients in the first few days following drug administration.

SUB ACUTE TOXICITY

It develops days to weeks[22] after completion of anthracycline therapy. Manifestations are:-Contractile dysfunction, Heart failure, Pericardial

effusion, Arrhythmias – VT , SVT. Insult to the myocardium can be early detected by monitoring serum Troponin I level [27] which may be used to predict the future development of ventricular dysfunction.

DELAYED TOXICITY

Chronic or delayed cardiotoxicity may manifests months or years after anthracycline therapy[3,27]. Commonest presentations:-Congestive heart failure, Dilated cardiomyopathy. Incidence – up to 20%.These produces progressive, irreversible myocardial damage which are fatal and results in cardiac death. Dilated cardiomyopathy is the most important long-term toxicity of doxorubicin [25].

CUMULATIVE DOSE RELATED CARDIOTOXICITY

Cardiotoxicity is mainly due to dose administered during each cycle and on total cumulative dose[28,]. The risk of anthracycline cardiomyopathy depends on cumulative dose. A 5% risk is seen at 400 to 450 mg/m^2 for doxorubicin [27], 900 mg/m^2 for daunorubicin, 800 to 935 mg/m^2 for epirubicin[29], and 223 mg/m^2 for idarubicin.

ECG changes found in 20 – 30 % of patients. Arrhythmias like SVT,VT are developed in 0.5 – 0.7 % of the patients. Serious arrhythmias like atrial fibrillation, atrial flutters are not common in acute toxicity. This shows more doxorubicin induced cardiac failure will occur in near future who are now in asymptomatic.

RISK FACTORS FOR CARDIOTOXICITY [26,]

- Cumulative dose - most significant risk factor
- Age – elderly more than 60 yrs and children up to 12 yrs
- Sex - females are more vulnerable [26]
- Length of infusion – risk more with rapid bolus infusion [26]
- Patients with abnormal cardiac function
- Mediastinal irradiation – prior or concomitant irradiation [30]
- Trisomy 21- higher risk of early clinical toxicity [32]
- Combination chemotherapy[24,25] with high dose cyclophosphamide, bleomycin,vincristine, mitoxantrone , pacletaxel etc.
- Obesity – more drug need because of more surface area
- Diseases – Hypertension, Diabetes mellitus, Coronary artery disease,

PROPOSED MECHANISMS OF DOXORUBICIN INDUCED CARDIAC TOXICITY [33,34]

- Oxidative stress [30]
  - Mitochondrial dependant ROS
  - NOS dependant ROS
  - NADPH dependant ROS
  - Fe – Doxorubicin complex
- Apoptosis

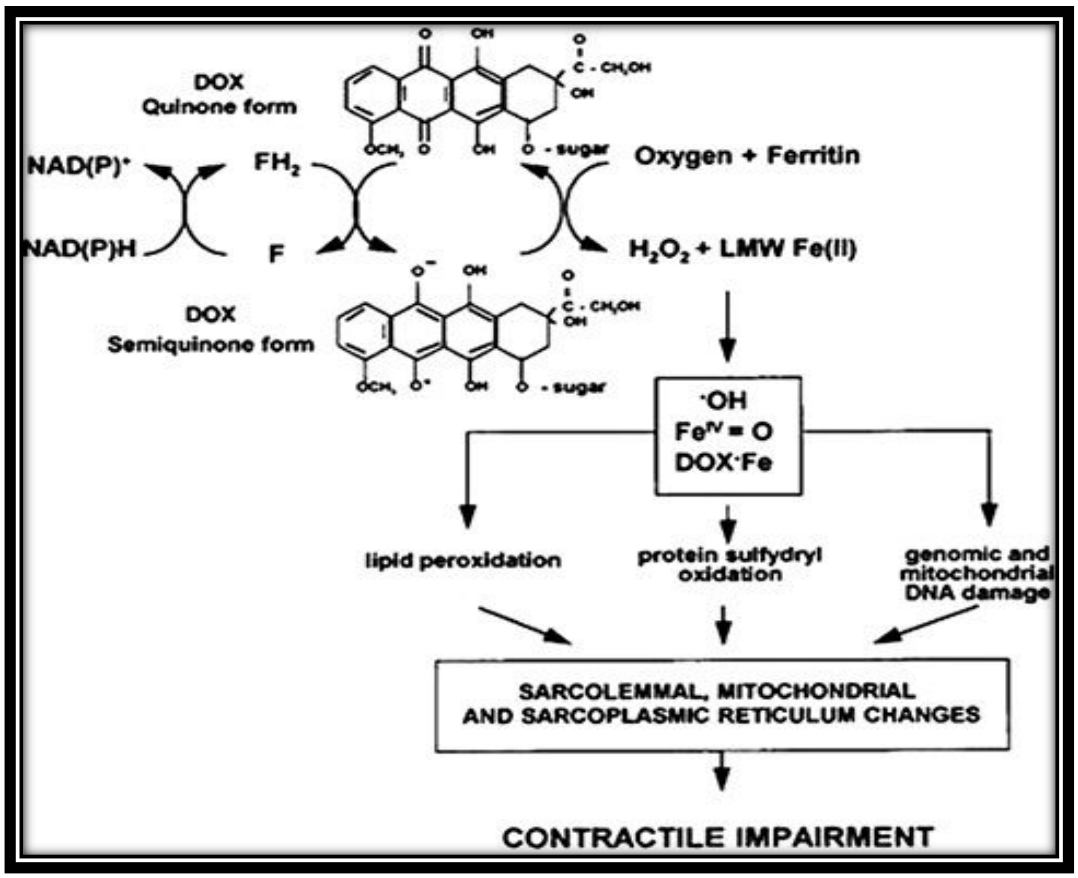
- Intracellular calcium dysregulation
- Changes in high energy phosphate pool
- Endothelin-1upregulation
- Extracellular matrix remodelling
- RAS Activation

Multiple mechanisms may involve in the formation anthracycline induced cardiomyopathy. Oxidative stress is the well accepted mechanism of the above all.

MITOCHONDRIAL DEPENDANT ROS[33]

Mitochondria produce more than 90% of ATP needed for cardiomyocyte. Doxorubicin is a cationic drug which forms an irreversible complex with cardiolipin and the complex sequestered in the inner mitochondria. Doxorubicin alters cardiolipin protein interface within electron transport chain leads to more superoxide formation[27,30]. Protein responsible for carnitine transfer within mitochondria also disrupted, which leads to mitochondrial dysfunction. Pathological changes found are mitochondrial swelling, Myelin figure formation.

Figure – 7 Mechanism of semiquinone formation



In an animal model of anthracycline induced cardiomyopathy showed that defect in long chain fatty acid oxidation, which coupled with excessive glucose metabolism within cardiac mitochondria. Development of congestive heart failure was mainly due to shift of aerobic metabolism into anaerobic one.

Sulaiman et al. found that doxorubicin disrupts mitochondrial gene expression and interfere with both nuclear and mitochondrial transcription regulation. Mn SOD is a free radical scavenger preserves mitochondrial function in doxorubicin cardiomyopathy. From this we can conclude that

mitochondria play a vital role in the development of doxorubicin cardiomyopathy. So preservation of mitochondrial function will lead to better cardiac outcomes.

NOS DEPENDANT ROS

Nitric oxide synthase is an enzyme with three isoforms. These are

- eNOS – Endothelial
- iNOS – Inducible
- nNOS – Neuronal

NOS has two domain

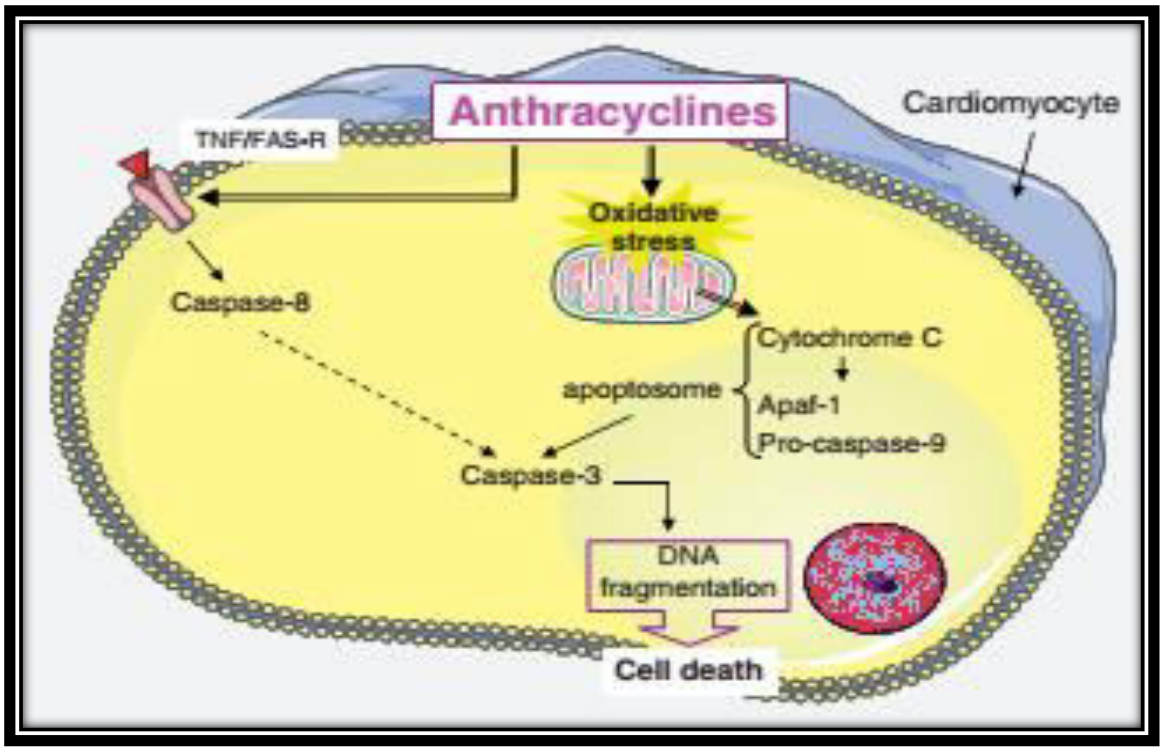
- Oxygenase
- Reductase

Doxorubicin binds to endothelial NOS reductase[33] and causes superoxide formation. Doxorubicin semiquinone formation mediated by eNOS by reducing one electron is a calcium independent reaction. Nitric oxide generates superoxide by eNOS if doxorubicin concentration increases. So doxorubicin induced apoptosis is mediated by eNOS. Uncoupling of eNOS leads to pressure induced heart failure. Role of iNOS is not well defined. So eNOS has an important role in doxorubicin induced cardiomyopathy.

NADPH dependant ROS[38]

Doxorubicin combines with NADPH and forms oxygen free radicals. Acute cardiotoxicity is associated with SNP in the p22phox and Rac2 subunits. Chronic toxicity is mediated by NADPH oxidase subunit NCF4.

Figure-8 Doxorubicin induced apoptotic cardiac cell death



Fe – Doxorubicin Complex[28,34]

Doxorubicin has strong affinity for ferrous iron and forms iron –doxorubicin complex which interacts with negatively charged membrane and produce lipid peroxidation. Reduction of doxorubicin with the help of free iron leads to free radicals generation. Doxorubicinol a metabolite of doxorubicin interacts with thiol group of membrane protein leads to cell damage.

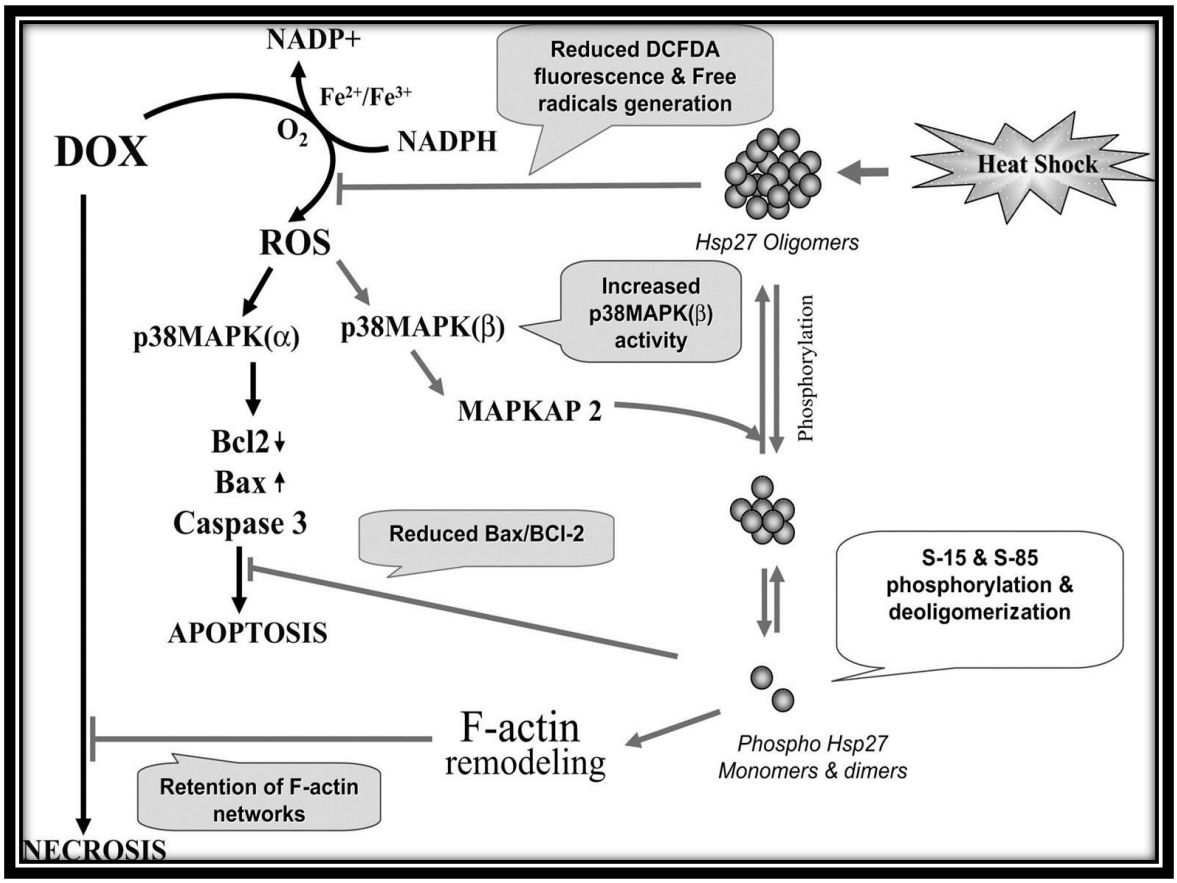
Doxorubicinol forms complex with thiol group of cytoplasmic aconitase or iron regulatory protein (IRP) and enhances stability of transferring mRNA & prevents its translation. Further decrease in IRP increases free iron level which promotes free radical generation. These free radicals interfere with iron sequestration and causes doxorubicin cardiomyopathy. ROS interferes with the function of G protein through lipid peroxidation. ROS alter the tertiary structure of the proteins. ROS also induces calcium release in myocardium.

APOPTOSIS[30,32]

Reactive oxygen species induce the formation of free radicals which stimulate proapoptotic genes and induce apoptosis via both intrinsic and extrinsic pathways, causes death of cardiac myocytes. In an apoptotic model, heat shock protein 1(HSP1) is activated by oxidative stress which promotes more HSP 25 protein production. By stabilizing p53 gene, HSP 25 promotes proapoptotic pathway. HSP acts as molecular chaperones which stabilize the proteins involved in antiapoptotic pathway by inhibiting their dephophorylation, ubiquination and its degradation[33]. By these mechanisms HSP 10,27,60 and Bcl-2 are maintains mitochondrial functions.

Apoptosis is induced by the activation of caspase-3. Nitric oxide donor, s-nitrosyl-N-acetyl penicillamine (sNAP) produces antiapoptotic effects by suppressing caspase activity in cardiomyocyte with doxorubicin.

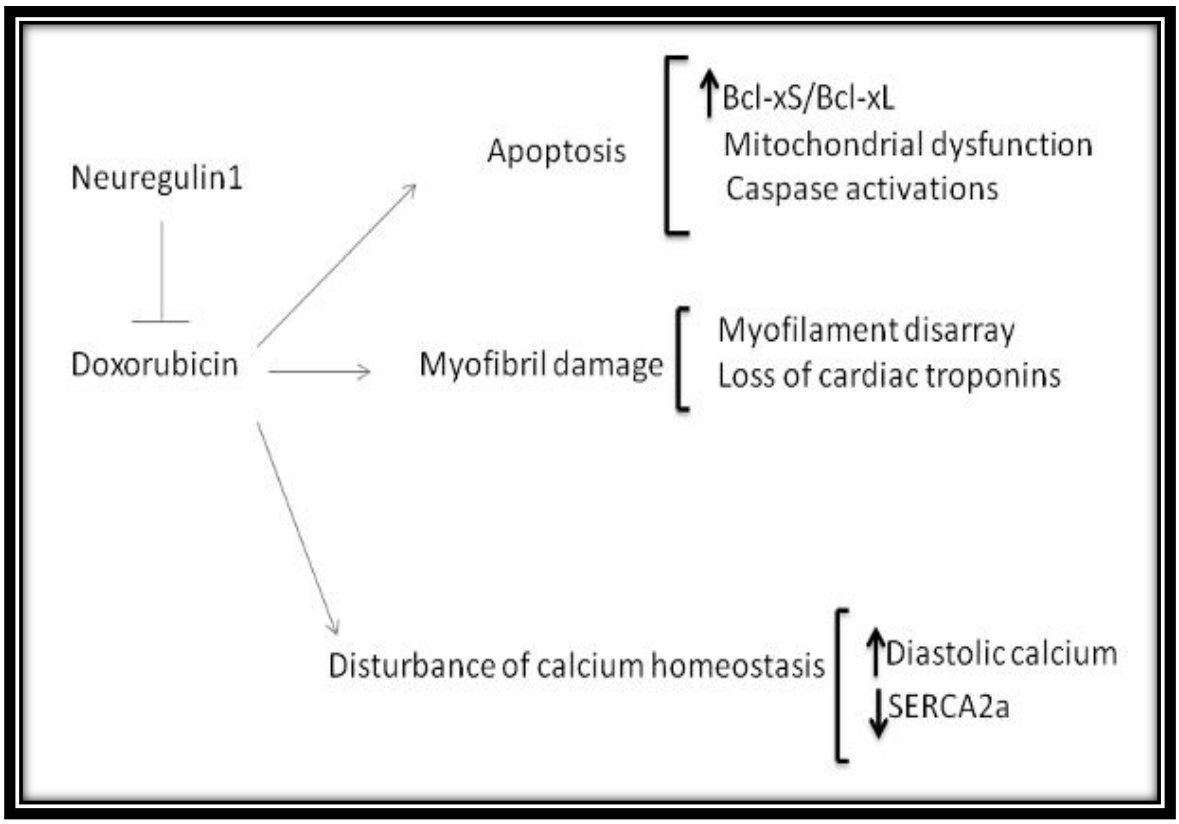
Figure – 9 Role of Heat Shock Proteins in Cardiotoxicity



INTRACELLULAR CALCIUM DYSREGULATION

It may be due to ROS production. Reactive oxygen species and hydrogen peroxide alter normal calcium homeostasis through disruption of sarcoplasmic reticulum in cardiac myocytes. This is mainly mediated by decreased SERCA2 mRNA expression which leads to poor calcium handling. It may also due to ryanodine receptor activation.

Figure – 10 Intracellular calcium dysregulation



Doxorubicin induces release of calcium from sarcoplasmic reticulum and opens the calcium channels. Doxorubicin increases L Type of calcium channel activity and inhibit sodium calcium exchanger channel in sarcoplasmic reticulum[39].

Calpain, a calcium dependent protease which is activated by calcium. Sarcoplasmic reticulum of cardiomyocyte contains more intracellular calcium. Oxidative stress induced by anthracyclines administration promotes calcium leakage, and activates calpain and cleavage of caspases-12. Doxorubicin increases sensitivity of mitochondria to the intracellular calcium.

MODIFICATIONS IN HIGH ENERGY PHOSPHATE POOL

Mitochondrial damage impairs the ability to generate adenosine tri phosphate (ATP). ATP depletion due to doxorubicin reduces the affinity of HSP 90 to ErbB2 which is a cardio protective protein. Because of ATP depletion, ErbB2 level also decreased. So HSP 90 unable to maintain its chaperone role. ATP depletion may be due to apoptosis and calcium dependent proteases which also consumes ATP.

Tokarska – schlattner et al[40]. demonstrated in an animal model of doxorubicin may impair energy signaling through AMP activated protein kinase (AMPK) which is involved in the inhibition of oxidation of fatty acid and to reduce mitochondrial function.

ENDOTHELIN-1 UPREGULATION[33]

Endothelin-1 enhances cell survival signaling in cardiac cells. Its level is up regulated in patients with congestive heart failure who is previously treated with doxorubicin. Bien et al. demonstrated in an animal model that endothelin receptor antagonist, bosentan decrease anthracycline induced cardiomyopathy with preserved myocardial contraction. Endothelin receptor antagonist decreases TNF-α and BAX expression, lipid peroxidation.

EXTRACELLULAR MATRIX REMODELING[33]

Anthracyclines inhibits matrix metalloproteinase 1(MMP-1) and its transcription in tumor cells. But in heart it has opposite effect by increasing MMP-2 and MMP-9 levels. This leads to weakening of collgenous matrix and further progress to pathological remodeling which is depend on NADPH level.

DOXORUBICIN INDUCED UPS ACTIVITY[34]

UPS is a proteolytic system which enhances the degradation and post translational modification of proteins. Anthracyclines activates UPS mediated proteolysis by act on proteosomes. Doxorubicin potentiates MAPKS, P38 and JNK which induces cardiomyocyte apoptosis by reduce the expression of antiapoptotic proteins like Bcl-2 and increases proapoptotic agents like Bax,caspase-3,caspase-9 etc.

ACTIVATION OF RENIN ANGIOTENSIN SYSTEM (RAS) & ACE ACTIVITY:-[41-43]

In animal models of doxorubicin induced cardiotoxicity, an increased Angiotensin Converting Enzyme activity was noted. Okumura et al[41].found that angiotensin converting enzyme (ACE) and chymase may involve in the production of doxorubicin induced cardiotoxicity in hamsters. Cardiac ACE activity was increased in doxorubicin induced cardiomyopathy hamsters.

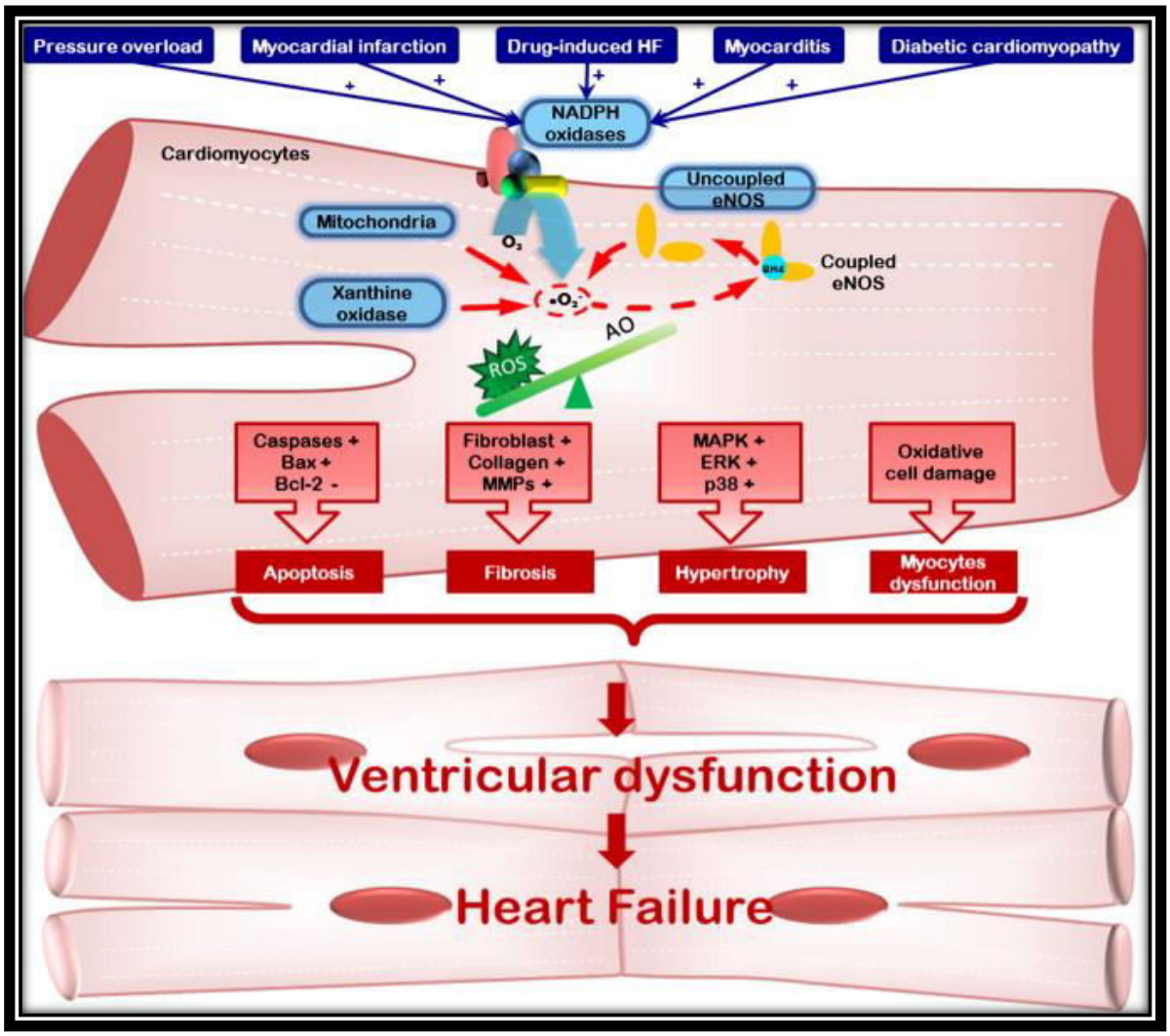
ACE inhibition significantly improved cardiac function and survival rate in hamsters. Lisinopril, treated hamsters improved mortality, cardiac remodeling and cardiac dysfunction in doxorubicin-induced cardiomyopathy suggest that ACE, plays a crucial role in the production of cardiomyopathy following the doxorubicin.

Toko et al[42]. found that AT1-mediated Ang II signaling pathway plays vital role in DOX-induced cardiac impairment. AT1 antagonist can be used to prevent DOX-induced cardiomyopathy. These results suggest that inhibition of the RAS in the heart may attenuate DOX-induced cardiac damage *via* a mechanism independent of blood pressure.Wen-na zong et al[59]. in their animal model of doxorubicin induced heart failure in rats explored that the changes in plasma level of angiotensin-(1–7)[Ang-(1–7)] and myocardial expression of angiotensin II type 1/2 receptors (AT1R / AT2R) and Mas receptor.

Tokudome et al[43].found that simultaneous administration of temocapril with doxorubicin in twenty male Sprague- Dawley rats protected against anthracyclines-induced cardiac damage. Myocardial tissue characterization was useful for the early detection of myocardial damage and the assessment of therapy. From these animal models data, RAS may also take part in anthracyclines cardiotoxicity.

Cardiac toxicity of anthracyclines may result from low levels of catalase in the heart combined with excessive mitochondria and myoglobin of the heart, which enhances the drug activation, as well as the sensitivity of cardiac glutathione peroxidase to free radicals attack. This destroys the activity of glutathione peroxides at the same time anthracycline administration stimulates cardiac hydrogen peroxide formation.

Figure – 11 Doxorubicin induced Heart Failure



In contrast anthracyclines can be easily activated to its intermediates by hepatic enzymes; the liver has more active free radical defense systems and is able to actively efflux anthracyclines and its metabolites. It may result from doxorubicin itself or its major metabolite, doxorubicinol. The leading hypothesis for doxorubicin induced cardiotoxicity involves oxidative stress induced by free radicals formation.

MONITORING

Treatment with anthracyclines may necessitate lifelong cardiac monitoring. General cardiac evaluation should be done to all the patients who are going to undergo doxorubicin therapy. Patients with cardiovascular risk factors need close and frequent monitor and keeping track on cumulative dose of doxorubicin[44].

Electrocardiogram[28]

Sinus tachycardia in previously normal heart rate is the earliest sign[34] of cardiotoxicity. Failure to return baseline heart rate, resting tachycardia, loss of respiratory variations to the heart rate are the important predictors of future cardiotoxicity.

Echocardiogram[44,46]

It Provides a wide spectrum of information on morphological and functional view of the heart & it is a non invasive method without Radiation exposure. Left ventricular ejection fraction (LVEF) and Fractional Shortening (FS) are commonly monitored in systolic dysfunction.

LVEF[44]:-

Baseline and serial measurement of LVEF by echocardiogram is standard protocol in patients on anthracycline treatment[44]. It is the most commonly used non invasive parameter to detect cardiotoxicity earliest.

$$LVEF = \frac{EDV\text{-}ESV \times 100}{EDV}$$

EDV – End Diastolic Volume

ESV - End Systolic Volume

Normal LVEF is between 50% - 70%. LVEF is calculated by 2D echocardiogram and Modified Simpson's formula. To decrease the inter-observer variability, echo done by same physician for each patient at different time points. Mild – Ejection fraction less than 45%, Intermediate – EF between 45 – 50% , severe toxicity EF < 45%.Stop doxorubicin therapy if LVEF decrease 15% from baseline value.

FRACTIONAL SHORTENING[36,46,:-

Calcuted from the following formula

$$FS = \frac{LVIDd - LVIDs \times 100}{LVIDd}$$

LVIDd **-** Left ventricular internal diameter in diastole

LVIDs - Left ventricular internal diameter in systole

Both LVEF and FS decrease in left ventricular systolic dysfunction[44].Reduction in LVEF and FS precedes development of CHF, if we stop further infusion of doxorubicin we can decrease further progression of heart failure.

All patients should have a baseline measure of LVEF. For doxorubicin, poor-risk patients should have a repeat study at 200 mg/m^2, and all patients should have a follow-up study at 300 to 400 mg/m^2 and every 50 to 100 mg/m^2 thereafter[28].

Radionuclide ventrculography[46]

Well established method to determine LVEF[35]. It can be used to determine regional wall motion and diastolic function by serial monitoring. It is an invasive procedure and exposure to radiation is limits its use routinely.

Cardiac biomarkers[46]

Troponin I ,T and Brain Natriuretic Peptide (BNP) are commonly used serological markers to detect early myocardial damage. B-type natriuretic peptide levels and troponin I levels, appear to hold the greatest promise as these are simple plasma markers and may correlate with subclinical left ventricular diastolic dysfunction.

Troponins[28,47]

Quantification of cardiac contractile proteins such as Troponin is a highly sensitive method of detecting myocardial cell injury and anthracyclines induced cardiotoxicity as well. Troponin I levels may be elevate in doxorubicin treated patients before deterioration in LVEF. Elevation of troponin I level after exposure to anthracyclines, an indicator of early cell death which may be beginning of ongoing process. Elevation of troponin I which correlate with subclinical LV Dysfunction.

Natriuretic peptide[27]

These are released from the atria (ANP), left ventricle (BNP) in response to circulating volume and intra cardiac pressures. BNP level correlates more with diastolic dysfunction than systolic dysfunction. Both troponin and BNP are highly specific and sensitive tool for early detection of myocardial defect.

MRI:-Valuable tool to assess myocardial function and damage

Computed tomography:-High radiation exposure, Low temporal resolution

Scintigraphy[46]**:-**Sensitive method to detect myocytes damage**.** Monoclonal antibody to cardiac myosin is tagged with I^{131}.

Endo Myocardial Biopsy (EMB)[28,30]

An invasive procedure, biopsy taken from endocardium of right ventricle of the heart. Gold standard investigation for anthracycline cardiomyopathy and provides histological evidence of cardiotoxicity. Total doses of doxorubicin as low as 250 mg/m^2 can cause pathological changes in the myocardium, as demonstrated by subendocardial biopsies. Histological changes may be found are mitochondrial swelling, Myofibrillar drop out, stellate scars, Vesicular dilatation, Adria cells, Myocytes hypertrophy, Myocytes degeneration and Loss of cross striations. Disadvantages are High cost, Need experts to do biopsy and to interpret its histology, only small sample of myocardium is tested.

STRATEGIES TO REDUCE DOXORUBICIN CARDIOTOXICITY

With awareness of the cardiac risk, anthracycline cardiotoxicity may be avoided by recognizing risk factors, early detection, limiting total cumulative dose, and more recently, using cardio protective agents or modified infusion drug regimens.

The prevention of doxorubicin induced cardiotoxicity depends on three approaches:

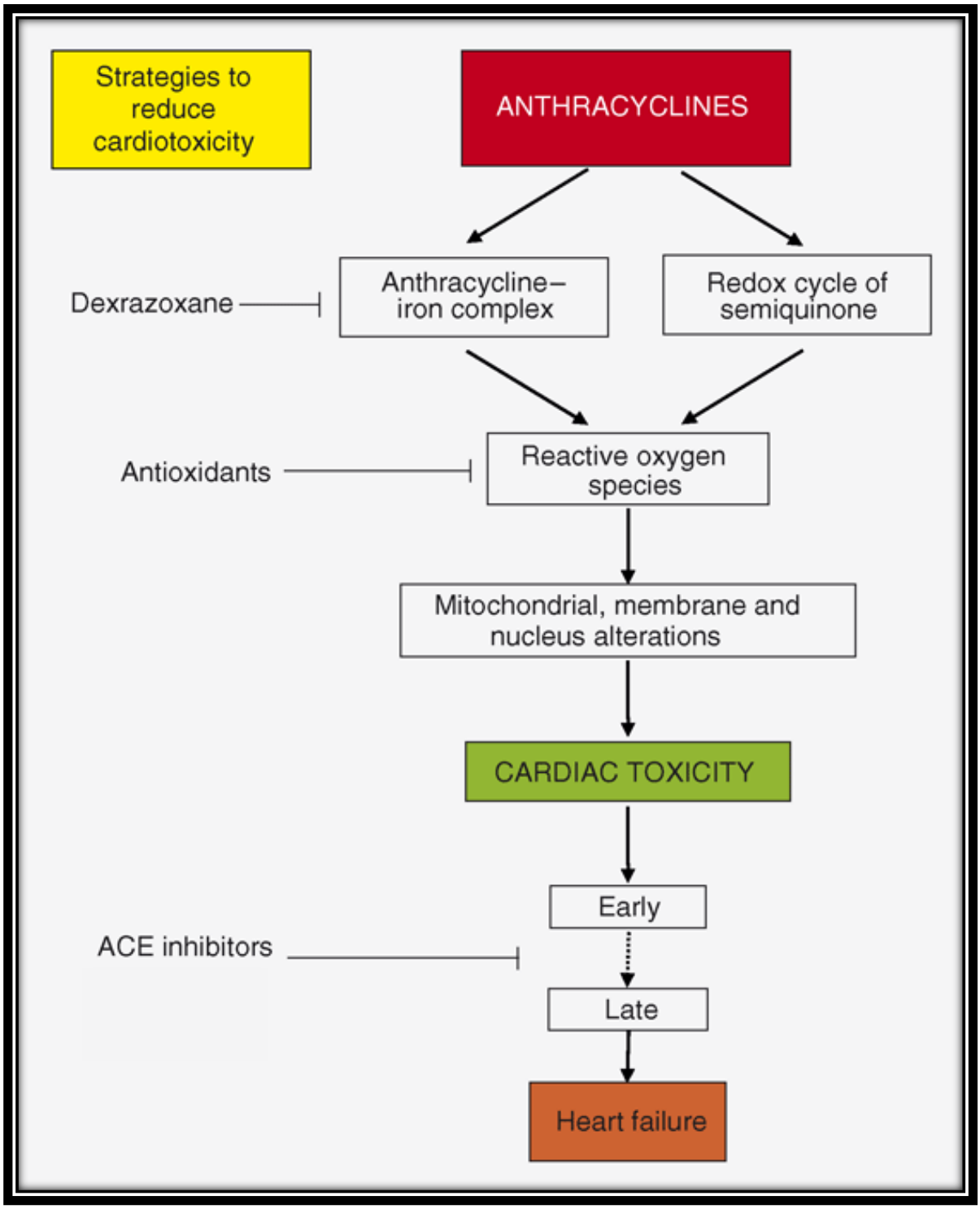
- Regular monitoring of cardiac function [28]

- Using analogues of anthracycline with less cardiotoxicity
- Modified administration of doxorubicin[30].

Doxorubicin toxicity can be minimized by:

- Changes in doxorubicin administration to continuous infusion.
- Minimizing the total cumulative dose to<400 mg/m^2
- Using liposomal - encapsulated doxorubicin[28].
- Using dexrazoxane to decrease the free iron formation. But it should be used only after exposure of doxorubicin dose of 300mg/m^2 because of the possibility of its ability to diminish the effect of anticancer agents.
- Using antioxidant to reduce the production of ROS with N-acetyl cysteine, coenzyme Q10.
- Treatment of early cardiac events needed, to minimize the evolution of doxorubicin-induced cardiotoxicity.
- ACE Inhibitors and β-blockers are routinely used to treat the late cardiac dysfunctions. ACE Inhibitors have been used to slow the progression of left ventricular dysfunction in many clinical conditions also in patients undergoing doxorubicin therapy. Hence early administration with enalapril seems to prevent the progression of late cardiotoxicity.

Figure – 12 Strategies to Reduce Doxorubicin Induced Cardiotoxicity



RISK FACTORS SCREENING AND PREVENTION OF CARDIAC EVENTS

All patients should be screened for preexisting cardiac risk factors like hypertension, diabetes, obesity and smoking before commencing therapy with doxorubicin. ACE inhibitors with or without beta blocker administration during the early course of the toxicity shows increased efficacy in the prevention of delayed cardiac events. Pretreatment with these agents can prevent the fall in ejection fraction seen with patient's undergone high-dose chemotherapy.

Dose limitation

The risk of cardiotoxicity can be reduced by keeping the total cumulative dose of doxorubicin well below550mg/m^2. It is recommended that the lifetime dose should be <450-550 mg/m^2 for doxorubicin. It is better to limit the doxorubicin cumulative dose less than 350 mg/m^2 in patients with cardiovascular risk factors which decrease the need of careful monitoring.

Schedule Modification[32]

Delayed cardiotoxicity is mainly related to peak doxorubicin concentration. Antineoplastic activity is more related to total drug exposure (Conc x AUC). A prolonged infusion of doxorubicin has been less

cardiotoxic than bolus injections. An important strategy to be followed to reduce the risk of cardiotoxicity is infusion over several hours. Weekly regimen schedule allowed more drug up to 200 mg/m^2 doxorubicin which may be tolerated by the individuals compared to 21 day routine cycle and also improve biopsy score. Continuous infusion (24-96 hrs) are safe by minimize the plasma drug concentration while maintaining antitumor effect which is confirmed by endomyocardial biopsy[44]. These measures reduce the cardiotoxicity of anthracyclines but did not prevent it. Rapid bolus infusion saline increased risk of extravasations / tissue necrosis. Using central line for prolonged >24 hrs infusion, would reduce the risk of extravasations.

Drawback of central line infusion

Increase the risk of infections, more time consuming to set up and flush the line weekly, line position checked by chest x ray, more than 96 hrs continuous infusions may leads to more chance of hand foot syndrome and mucositis, need of portal infusion pump and indwelling catheters.

ALTERNATIVE STRATEGIES

Liposomal Anthracyclines[28,44]

Liposomal formulations have been introduced to enhance the therapeutic index of free doxorubicin by encapsulation of doxorubicin within a macromolecular vector, such as a liposome. It permit more specific organ targeting of doxorubicin, less volume of distribution, less diffusion and less toxicity for healthy tissues .

Formulations of liposomal anthracyclines[18]

- Pegylated liposomal doxorubicin
- Non pegylated liposomal doxorubicin
- **Other unique delivery systems :-** Starch microspheres, Albumic microspheres, Lipeodal Doxorubicin

FDA recently approved liposomal formulations of doxorubicin for Kaposi sarcoma, carcinoma ovary, breast and Multiple Myeloma. These formulations have same efficacy with minimal cardiac toxicity when compared with free doxorubicin. Less chance of developing cardiotoxicity with pegylated liposomal doxorubicin than with normal one in all subgroups analyzed (age>65 yrs, prior anthracyclines therapy). However they may cause hand–foot syndrome and Stomatitis.

Non-pegylated liposomal doxorubicin is less cardiotoxic than conventional doxorubicin. It has comparable antitumor activity with less diarrhea and nausea/vomiting, neutopenia. Findings from a meta-analysis showed a lower rate of clinical and subclinical heart failure with nonpegylated liposomal doxorubicin.

Hence Liposomal doxorubicin should be used in patients with metastatic breast cancer who are at high risk. Nonpegylated liposomal doxorubicin is more efficacious and less cardiotoxic over free doxorubicin for patients treated adjuvant with anthracyclines iron-chelating agents.

Anthracycline analogues[27]

Analogue development holds the promise of good antitumor activity with less cardiotoxicity. Epirubicin produce heart failure only in 10% of breast cancer patients took cumulative dose of more than 600mg/m^2.

Idarubicin

The dose of idarubicin is 12 mg/m^2/day for 3 days by intravenous injection. Slow injection over 10-15 minutes is followed to avoid extravasations. It has less cardiotoxicity than doxorubicin.

Daunorubicin

Daunorubicin (rubidomycin, daunomycin) is infused intravenously. The dose is 25-45 mg/m^2/day for 3 days. Total doses of >1000 mg/m^2 are associated with risk of cardiotoxicity.

Epirubicin

It is indicated in adjunctive therapy for treatment of breast cancer. It is administered in doses of 100-120 mg/m^2 intravenously every 3-4 weeks. Total doses >900 mg/m^2 sharply increase the risk of cardiotoxicity. Its toxicity profile is the same as that of doxorubicin.

Valrubicin

Valrubicin is a semi-synthetic analog of doxorubicin, used exclusively for intravesicular treatment of bladder cancer. 800mg are instilled into the

bladder once a week for 6 weeks. Less than 10% of instilled drug is absorbed systemically.

REGIMENS WITHOUT ANTHRACYCLINES[44]:-

Another strategy to reduce cardiotoxicity is simply not to use doxorubicin. Although taxanes also have a potential cardiovascular risk, in a comparative study found that treatment with docetaxel conferred no apparent cardiotoxicity benefit than doxorubicin. Thus, the choice of agent than doxorubicin should be balanced against reduction in antitumor efficacy and against the overall toxicity profile of the two agents. Cardiac events occur during doxorubicin schedule or in the first year after its completion is often manifesting as arrhythmias, such as atrial fibrillation or pericarditis. These should be treated according to guidelines, which may heart rate or rhythm modifying drugs and antithrombotic agents. Patients with symptomatic CHF should receive ACE inhibitors and beta blockers.

Blocking agents[44]

Differences in the biochemical mechanisms of antitumor and cardiotoxic activity provide a potential avenue of selectively inhibiting or preventing the adverse effect.

Targets for the approach

- Agents that prevent free radicals generation
- Salvage free radicals.

FREE RADICALS[50]

Oxidative Free radicals are constantly formed in the human system and has been implicated in several human diseases. These free radicals are scavenged or removed by enzymatic and non enzymatic antioxidant defense mechanism. When these defense mechanisms are inadequate, oxidative stress can damage proteins, carbohydrates, lipids and nucleic acids.

A free radical contains one or more unpaired electrons in their orbitals. When two radicals meet, they can combine their unpaired electrons and join to form a covalent bond

$$A^{O} + A^{O} \text{---------- } A \text{ ---} A$$

Types of free radicals

Hydroxyl radicals (*OH)

It is one of the most reactive free radical, important role in tissue damage by radiation.*OH radical causes DNA fragmentation and extensive alterations in the purine and pyrimidine bases. Hydroxyl radicals (*OH) are also known to induce lipid peroxidation of fatty acid side chains of membrane phospholipids. Accumulation of lipid peroxides in a biological membrane disrupts its integrity and function.

Superoxide radicals

$$O_2 + e^{-} \text{---------- } O_2^{\cdot -}$$

Superoxide radicals ($O_2^{\cdot -}$) is an oxygen molecule deficient of one electron, involved in the regulation of fibroblast proliferation, vasodilatation and phagocytosis.H_2O_2 is not a free radical but is a powerful oxidizing agent and is removed by the enzymes catalase and glutathione peroxidase.

Nitric oxide

NO has one unpaired electron and can be regarded as free radical. Combination of NO with superoxide radicals results in the formation of peroxynitenite (OONO) which is highly toxic.

Transition metal ions and free radicals

Iron and copper ions are excellent promoters of free radical formation. These can convert H2O2 in to highly reactive *OH , leads to accumulation of cytotoxic end products of lipid peroxides.

Antioxidant defensive mechanisms

Antioxidant or free radical scavenger is a substance at low concentrations can prevent or delay the oxidation of an oxidizable substrate.

Major intracellular Antioxidants[50]

SOD, Catalase, Glutathione peroxidase, α Tocopherol, Ascorbic acid

Major Extracellular Antioxidants

Uric acid**,** Bilirubin**,** Ceruloplasmin**,** Albumin**,** α Tocopherol, Ascorbic acid

OXIDATIVE STRESS AND DISEASE[50]

Disease involving several organs / systems

Rheumatoid arthritis, Myocardial infarction, Malignancy and Aging

Disease involving specific organs / systems

Nervous system:- parkinsonism, Alzheimer's disease; ***Renal system*:-** heavy metal, aminoglycoside & NSAIDs toxicity; ***Respiratory system*:-** emphysema, oxygen toxicity; ***Endocrine* :-** diabetes mellitus; ***Drug toxicity*:-** Anthracyclines, halothane, chloroform, Lead poisoning, Antimalarials – Haemolysis.

ANTIOXIDANTS OF NATURAL ORIGIN

Superoxide dismutase (SOD)

The enzyme superoxide dismutase (SOD), found in all cells and is necessary for scavenging the Superoxide radical and prevent its accumulation and production of oxidative stress to the living system. SOD catalyzes a dismutation reaction, where by one $O_2^{\cdot -}$ is oxidized to O_2 and the other reduced to H_2O_2.

$$O_2 + O_2^{\cdot -} + 2H \text{ ---------- } H_2O_2 + O_2$$

It protect against ischemic or reperfusion injury seen after MI, cerebral stroke or organ transplants. The large inflow of oxygen during reperfusion results in generation of $O_2^{\cdot -}$ and H_2O_2 which can be scavenged by SOD.

Alpha Tocopherol

Severe neurological damage, hemolytic syndrome, atherosclerosis, retrolental fibroplasias.

Ascorbic acid

Vitamin c is an essential component of human diet and functions as a cofactor of several hydrolyze enzymes. Useful in atherosclerosis and cancers.

Physiological antioxidants[50]

Lactoferrin – iron binding protein**,** Nicotanamide**,** Glutathione – organ transplantation, cystic fibrosis, AIDS- p.carnii pneumonia**,** Acetylcysteine – paracetamol poisoning**,** Beta carotene – anti cancer effect**,** Melatonin.

Synthetic antioxidants:- Allopurinol , Desferrioxamine, Probucol.

Drugs with additional antioxidant property[50]

ACE Inhibitors, Calcium channel blockers, NSAIDs, Selegiline.

Free radical scavengers[50]

- Vitamin E (α Tocopherol),N-Acetylcysteine, Coenzyme Q10, Phrenylamine, Dexrazoxane and ACE Inhibitors.

DEXRAZOXANE[8,49]

Dexrazoxane is a prodrug of bisdioxopiperazine derivative. It is an iron chelating agent. Highly effective in reducing doxorubicin induced cardiotoxicity and extravasations injury. It is the only FDA approved drug to prevent doxorubicin induced cardiotoxicity. Intracellularly it is hydrolyzed to form bidentate chelator. Structurally it is similar to EDTA also a strong inhibitor of topoisomerase II enzyme. It quickly binds to intra cellular iron and strips Fe^{2+} from iron – doxorubicin complex by which it decrease the generation of free radicals[8]. Although its benefits against late cardiotoxicity not proved, but it is known to decrease the acute cardiotoxicity of doxorubicin. Dexrazoxane also have decrease antitumor response rate to the anthracycline treatment. Route of administration – intravenous. Biphasic elimination – distribution $T_{½}$ - 0.8 hrs, elimination $T_{½}$ -9 hrs. Though dexrazoxane may be useful to reduce cardiotoxicity; its use should be weighed against the risk of a lower response rate[49] and the additional costs of treatment.

RENIN AND ANGIOTENSIN SYSTEM (RAS)

Pathophysiology of heart failure, hypertension, myocardial infarction, diabetic nephropathy and chemotherapy induced cardiotoxicity are mediated by the renin–angiotensin system (RAS). There are two forms of Angiotensin identified, Angiotensin I [AngI]) & Angiotensin II [AngII]) derived by cleavage of AngI by an enzyme termed Angiotensin-converting enzyme (ACE). The AngII is to be the more active form[53].

Components of the Renin–Angiotensin System[52]

AngI derived from the angiotensinogen with the help of renin. ACE convert AngI to produce the AngII which in turn act on AT1 and AT_2 receptors. An alternative ACE independent pathway also involved in AngII synthesis. Biologically active Angiotensin peptides (AngIII, AngIV, Ang[1–7]), Angiotensin binding receptors AT_1, AT_2, AT_4, Mas are involve in cellular hypertrophy, fibrosis, inflammation and apoptosis.

Short -loop negative feedback[52] :-Increased renin stimulates the formation of AngII, which in turn stimulates AT_1 receptors on juxtaglomerular cells to inhibit renin release.

Long-loop negative feedback[52]:- an AngII-induced increase in blood pressure inhibits renin release. ACE inhibitors, Angiotensin receptor

blockers (ARBs), and renin inhibitors interrupt both the short- and long-loop negative feedback mechanisms and therefore increase renin release.

Angiotensin converting enzyme:-

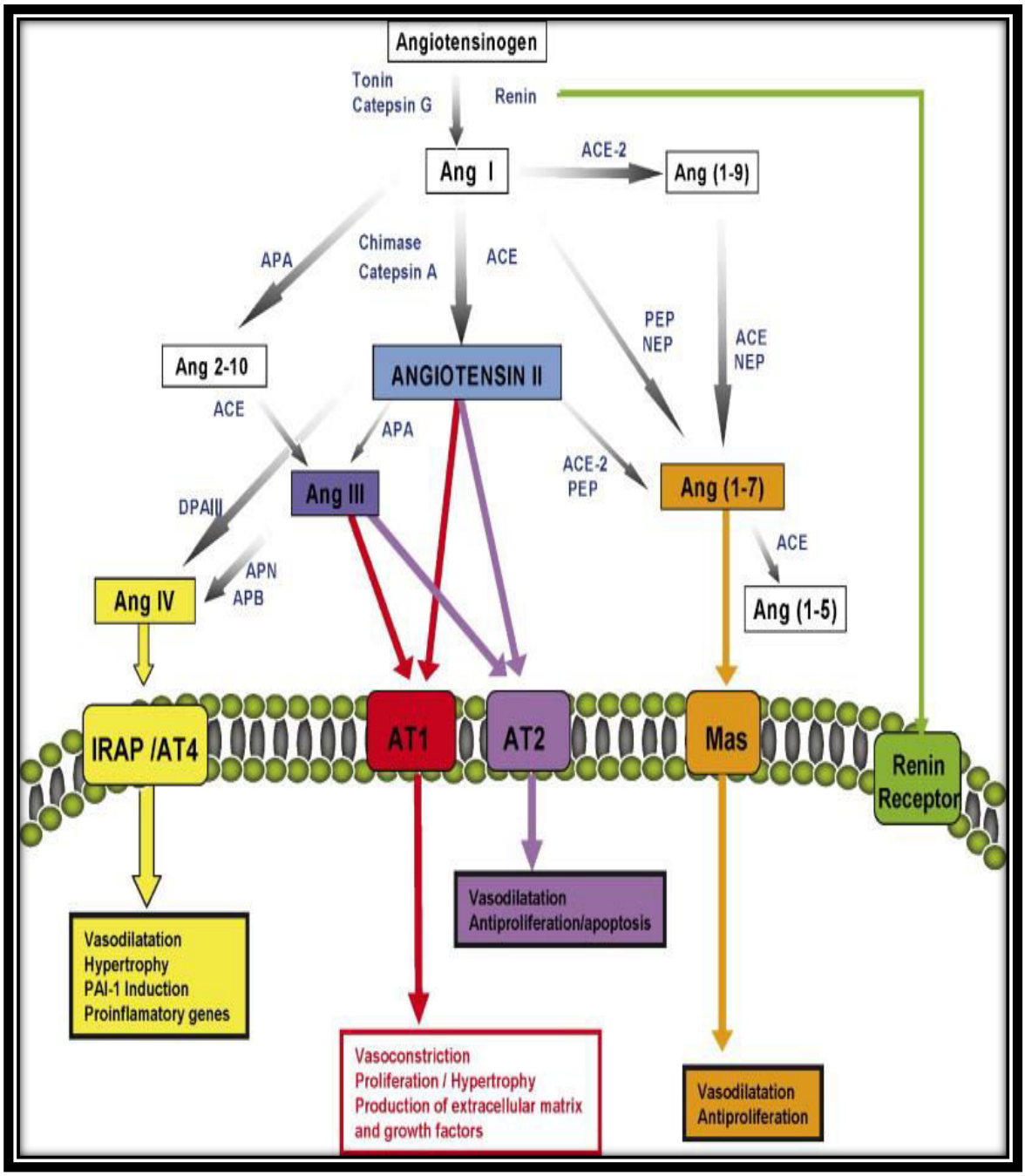
Angiotensin Converting Enzyme (kininase II, Dipeptidyl carboxypeptidase)is an ectoenzyme which is identical to kininase II, the enzyme that inactivates bradykinin and other potent vasodilator peptides.

Angiotensin-Converting Enzyme 2

ACE2 cleaves one amino acid from the carboxyl terminal to convert AngI to Ang(1–9) and AngII to Ang(1–7). It act as a counter-regulatory mechanism to oppose the effects of ACE. ACE2 regulates the levels of AngII and limits its effects by converting it to Ang(1–7), which binds to Mas receptors and elicits vasodilator and anti-proliferative responses.ACE2 is not inhibited by the standard ACE inhibitors and has no effect on bradykinin.

Angiotensin(1-7)[54] :- Angiotensin(1-7) is formed by many pathways. Ang(1–7) opposes many of the effects of AngII: causes vasodilatation, NO production, potentiates the vasodilator effects of bradykinin, has anti-angiogenic, anti-proliferative & cardio protective in cardiac ischemia and heart failure. These effects are modulated through Mas receptor.

Figure – 13 Renin Angiotensin System and its Physiological action on cardiovascular system[52]



Angiotensin Receptors[53]

AT_1 receptors stimulate the membrane-bound NADH/NADPH oxidase which generates reactive oxygen species (ROS). ROS may contribute to biochemical effects and physiological effects. The AT_2 receptors may counterbalance many of the effects of the AT_1 receptors.

Functions and Effects of the Renin–Angiotensin System[53,54]

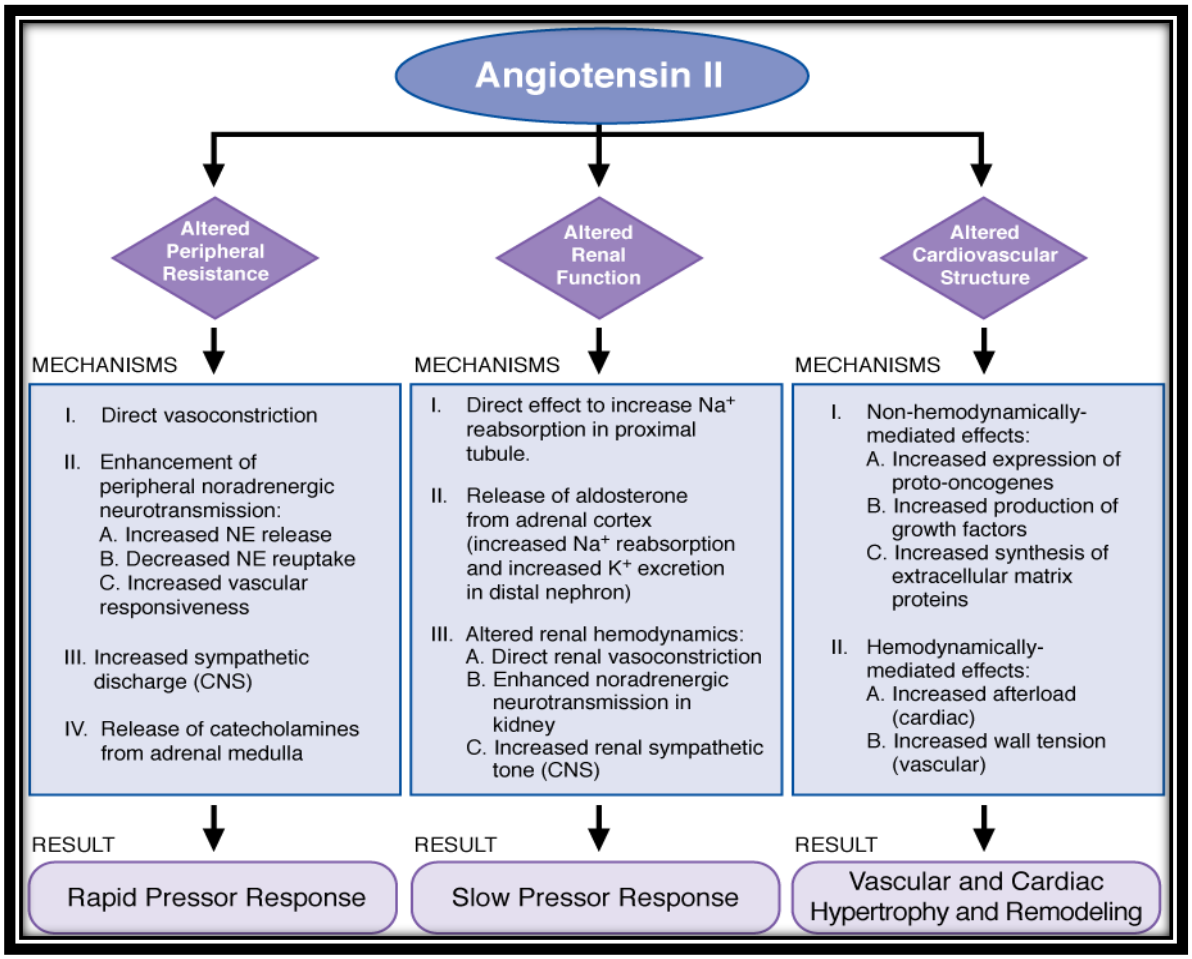
The main effects of AngII on the cardiovascular system include: fast pressor response, slow pressor response, Vascular and Cardiac hypertrophy and remodeling. This rapid pressor response to AngII is due to rise in total peripheral resistance. AngII stimulates the synthesis of endothelin-1 and superoxide anion, which may contribute to the slow pressor response. AngII stimulates the cardiovascular remodeling, vascular and cardiac cells hypertrophy and excessive synthesis and deposition of collagen by cardiac fibroblasts.

Cardiovascular Hypertrophy and Remodeling[53]

These effects of AngII are mediated by acting directly on cells to induce the expression of specific proto-oncogenes (c-*fos*, c-*jun*, c-*myc*, and *egr*-1) that alter the expression of growth factors such as basic FGF, PDGF, and TGF- β. In addition, changes in cardiac preload (volume expansion owing to

Na^+ retention) and afterload (increased arterial blood pressure) probably contribute to cardiac hypertrophy and remodeling.

Figure – 14 Angiotensin II mediated Pathophysiology on Heart[52]



INHIBITORS OF THE RENIN–ANGIOTENSIN SYSTEM[54]

Three types of inhibitors of RAS system are utilized therapeutically

- ACE inhibitors (ACEIs)
- Angiotensin receptor blockers (ARBs)
- Direct renin inhibitors (DRIs)

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

History[52]

In the 1960s, Ferreira and colleagues found that of pit viper venoms have factors that intensify vasodilator responses to bradykinin. These bradykinin-stimulating factors are a family of peptides that inhibit kininase II, an enzyme that inactivates bradykinin. Erdös and coworkers found that kininase II and ACE are the same enzyme, which catalyzes both the synthesis of AngII and the destruction of bradykinin. It decreased blood pressure in many patients with hypertension and beneficial effects in patients with heart failure.

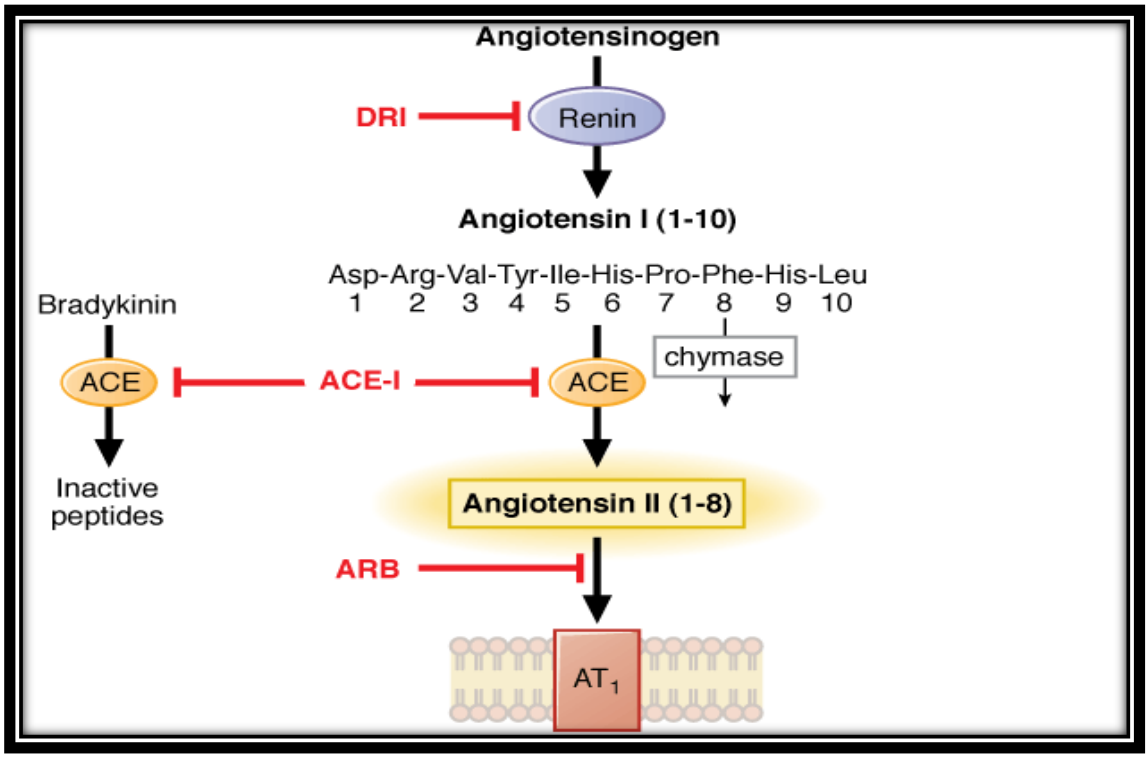
CLASSIFICATION OF ACE INHIBITORS[53]

Sulfhydryl- ACE inhibitor – Captopril

Phosphorus - ACE inhibitors – Fosinopril

Dicarboxyl- ACE inhibitors - Enalapril, Ramipril, Lisinopril, Quinapril, Benazepril, Perindopril Moexipril, Trandolapril.

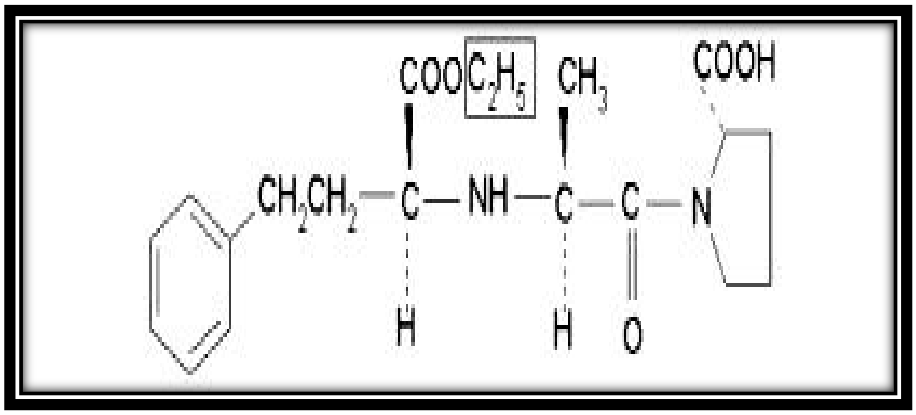
Figure – 15 Agents interferes with RAS System[53]



Captopril and lisinopril are active drugs. All other are prodrugs. These prodrugs are inactive until converted to their corresponding di-acids. Kidney is the primary root of excretion in most of the ACE inhibitors. Patients with poor renal function diminish the plasma clearance of these agents and doses should be decreased in patients with renal impairment[55]. Initial dosages of all ACE inhibitors should be reduced in patients with high plasma levels of renin (e.g., patients with heart failure and salt-depleted patients). All ACE inhibitors have similar therapeutic indications, adverse-effect profiles.

ENALAPRIL

Figure – 16 Chemical structure of Enalapril



Enalapril is an orally active ACE Inhibitor without Sulfhydryl group[53] has longer duration of action than Captopril. Enalapril maleate is a prodrug which is hydrolyzed in the liver to enalaprilat. Enalaprilat is a powerful inhibitor of ACE. Enalapril is absorbed rapidly orally with an oral bioavailability of ~60%. Although peak plasma concentrations of enalapril occur within an hour, enalaprilat peak concentrations achieves only after 3-4 hrs. Enalapril has a half life of $t_{1/2}$ ~1.3 hours, but enalaprilat plasma $t_{1/2}$ of ~11 hours because of its tight binding to ACE. Enalapril is eliminated by the kidneys. The dosage of enalapril ranges from 2.5-40 mg daily (single or divided dose), with 2.5 and 5 mg daily being appropriate for the initiation of therapy for heart failure and hypertension, respectively[53].

Pharmacology

Enalapril is inactive until de-esterifies into enalaprilat, a specific competitive inhibitor of ACE. ACE Inhibitors reduces plasma Angiotensin II levels and as a result of loss of feedback inhibition, plasma rennin activity increases. Plasma aldosterone may be reduced, which can increases plasma potassium. ACE inhibition may increase bradykinin level, a potent vasodilator and stimulate prostaglandins, EDRF, NO. Enalapril in single dose of 2.5 – 20 mg blunts the BP response to injected Angiotensin I for more than 4 hrs while plasma ACE activity decreased up to 24 hrs. Plasma renin activity increases while that of Angiotensin II and aldosterone decrease. The BP fall is due to peripheral vasodilatation without reflex tachycardia. Bradykinin is degraded by peptidyl dipeptidase also known as ACE, some of the effects of ACE inhibition may be due to elevated bradykinin.

Pharmacokinetics

After oral intake of 10 mg of enalapril reaches peak level in 1 hr but its active form enalaprilat peaks at 4 hrs[52]. Oral absorption $\geq$ 60%, absorption is not affected by food. Bioavailability is 40%. Urinary excretion is about 70%. Plasma half life of Enalapril is short, but for Enalaprilat is 35 hrs. Plasma binding protein (Enalaprilat) is 60%.

Metabolism

In vivo enalapril rapidly converted into enalaprilat. There is no evidence of further metabolism because 94% of orally administered enalapril is recovered as either enalaprilat or its di- acid.

THERAPEUTIC USES OF ENALAPRIL[52-55]

Enalapril that interfere with the RAS play a prominent role in the treatment of cardiovascular disease.

- **Hypertension**- ACE inhibitors monotherapy decrease the blood pressure in ~50% of patients with moderate hypertension. 90% of patients with mild to moderate hypertension will be controlled by the combination of an ACE inhibitor and either a Ca^{2+} channel blocker, a β adrenergic receptor blocker, or a diuretic.
- **Acute Myocardial Infarction** - The beneficial effects of ACE inhibitors in acute myocardial infarction are particularly large in hypertensive and diabetic patients. In high-risk patients (e.g., large infarct, systolic ventricular dysfunction), ACE inhibition should be continued for long term.
- **Diabetes Mellitus and Renal Failure** - Reno protection in type 1 diabetes, as defined by changes in albumin excretion, and are independent of blood pressure reduction. In addition, ACE inhibitors

may decrease retinopathy progression in type 1 diabetics and minimize the progression of renal insufficiency.

- **Renal protection** - By lowering arterial blood pressure and by dilating renal efferent arterioles, ACE inhibitors reduce this glomerular injury.
- **Scleroderma Renal Crisis** - improves survival of patients with scleroderma renal crisis.
- **Left Ventricular Systolic Dysfunction and CHF**[52]

ACE inhibitors should be started in all asymptomatic & symptomatic patients with impaired left ventricular systolic function. The more severe the ventricular dysfunction, the greater the benefit from ACE inhibition.

- Prevents or delays the progression of heart failure
- Decreases the incidence of sudden death and myocardial infarction,
- Decreases hospital admission
- Improves quality of life.

Inhibition of ACE commonly

- Reduces afterload and systolic wall stress
- Increase both cardiac index and cardiac output
- Improve indices of stroke volume.

In systolic dysfunction, AngII decreases arterial compliance, and this is reversed by ACE inhibition. Heart rate & Systemic blood pressure falls, Reno vascular resistance falls sharply, and renal blood flow increases. Natriuresis occurs as a result of the improved renal hemodynamic, the reduced stimulus to the secretion of aldosterone by AngII, and the diminished direct effects of AngII on the kidney. The excess volume of body fluids contracts, which reduces venous return to the right side of the heart. A further reduction results from venodilation and an increased capacity of the venous bed.

The response to ACE inhibitors also involves reductions of pulmonary arterial pressure, pulmonary capillary wedge pressure, and left atrial and left ventricular filling volumes and pressures. Consequently, preload and diastolic wall stress are diminished. The better hemodynamic performance results in increased exercise tolerance[52] and suppression of the sympathetic nervous system. Cerebral and coronary blood flows usually are well maintained, even when systemic blood pressure is reduced.

In heart failure, ACE inhibitors reduce ventricular dilation and tend to restore the heart to its normal elliptical shape. ACE inhibitors may reverse ventricular remodeling via changes in preload/afterload, by preventing the growth effects of AngII on myocytes, and by attenuating cardiac fibrosis induced by AngII and aldosterone[55].

ACE Inhibitors and Survival in CHF[52]

Independent of etiology ACE inhibitors improves survival in patients with CHF due to systolic dysfunction. CONSENSUS Trial demonstrated a 40% mortality reduction after 6 months of enalapril therapy in severe CHF, while others have shown that enalapril also improves survival in patients with mild-to-moderate CHF. ACE inhibitors slow the development of symptomatic CHF in asymptomatic LV dysfunction.

ADVERSE EFFECTS OF ACE INHIBITORS

In general, ACE inhibitors are generally well tolerated group of drugs. They rarely produce metabolic side effects on long-term therapy. Improves insulin sensitivity and reduce the cholesterol and lipoprotein (a) levels in proteinuric patients.

Hypotension- In patients with elevated PRA fall in blood pressure can occur following the first dose of an ACE inhibitor.

Cough - ACE inhibitors produce dry cough in 5-20% of patients[54] due to the accumulation of bradykinin, substance P, prostaglandins in the lungs. **Hyperkalemia[53] -** ACE inhibitors may produce hyperkalemia in patients with renal failure, diabetic patients or in patients taking K^+-sparing diuretics, K^+ supplements, β adrenergic blockers, NSAIDs. However K^+ retention is rarely encountered in patients with normal renal function.

Acute Renal Failure - ACE Inhibition may precipitate acute renal failure in patients with history of bilateral renal artery stenosis, renal artery stenosis of a single remaining kidney, congestive heart failure, or hypovolemic state due to diarrhoea or diuretics.

Teratogenic Potential[55] - ACE inhibitors should be discontinued as soon as pregnancy is confirmed. Pregnant women exposed to ACEI associated with Pulmonary hypoplasia, IUGR, Premature labour, Bony malformations and Limb contractures.

Angioedema - ACE inhibitors may induce a rapid swelling in the nose, larynx, lips, throat, mouth, glottis, and tongue in less than 0.5% of patients.

Other Side Effects

- Dysgeusia (an alteration in or loss of taste), Skin rash
- Neutopenia (symptoms include sore throat and fever)[54]
- Glycosuria, Hepatotoxicity.

CONTRAINDICATIONS

- Known hypersensitivity to ACE inhibitors
- Angioneurotic edema relating to previous ACEI therapy
- Bilateral renal artery stenosis[55]
- Stenosis to a single solitary kidney[53]

- Aortic stenosis or outflow tract obstruction

DRUG INTERACTIONS

- Bioavailability of ACE inhibitors decreased by antacids.
- ACE inhibitor–induced cough is worsened by capsaicin.
- NSAIDs reduce the antihypertensive response to ACE inhibitors by blocking PG synthesis
- NSAIDs and ACEI combination precipitates nephrotoxicity.
- K^+-sparing diuretics & K^+ supplements may aggravate ACE inhibitor–induced hyperkalemia[54].
- ACE inhibitors increase plasma digoxin and lithium concentrations
- ACE inhibitors increase hypersensitivity reactions to allopurinol.
- Diuretics accentuates hypotensive action of ACEI[53].

ACE INHIBITORS AND OXIDATIVE STRESS[51,60]

ACE inhibitor like enalapril has important role in prevention of oxidative stress in vascular smooth muscles. Cells of smooth muscle, endothelium, and fibroblasts are using NADH or NADPH as a substrate for the formation of superoxide anion in response to Angiotensin II[60] activation. Therefore, superoxide production may be increased in conditions where renin angiotensin system is activated. Atherosclerosis is one of the risk factor for heart disease in which ACE and vascular NAD(P)H oxidase

activity is increased. Progression of atherosclerosis mediated cardiac events mainly due to NAD(P)H oxidase activation and increased level of superoxide within the vascular wall. Combination of Superoxide with NO is much faster than the superoxide dismutation reaction. Peroxynitrite is produced by this reaction shunting NO from its typical targets like vasodilatation and platelet inhibition. Thus, reduction of NO bioactivity and promotion of oxidative stress in the blood vessels are mainly due to the dual effect of vascular super oxides.

Superoxide has an important role in the development of cardio vascular disease because of NADPH oxidase overactivity[61]. Hydrogen peroxide (H_2O_2) is produced by superoxide dismutase associated with lipid oxidation with the help of transition metals. Apoptosis & necrosis in cardiovascular cells are induced by H_2O_2 over activity.

Proliferation of smooth muscle cell is stimulated by H_2O_2. H_2O_2 also inhibits endothelial cells proliferation. H_2O_2 increases the expression of endothelin-1 and activates of proapoptotic pathway in endothelial cells. Smooth muscle hypertrophy and hypertension are mainly mediated by NAD(P)H oxidase activation in response to Angiotensin II .

ACE Inhibition as an Antioxidant Strategy

Chopra et al[51].described about antioxidant effects ACE inhibitors in his experimental model. He quoted that free radical and oxidant scavenging effects are depends on Sulfhydryl group. Sulfhydryl - and nonsulfhydryl containing ACE inhibitors like enalapril inhibits the lipid peroxidation.

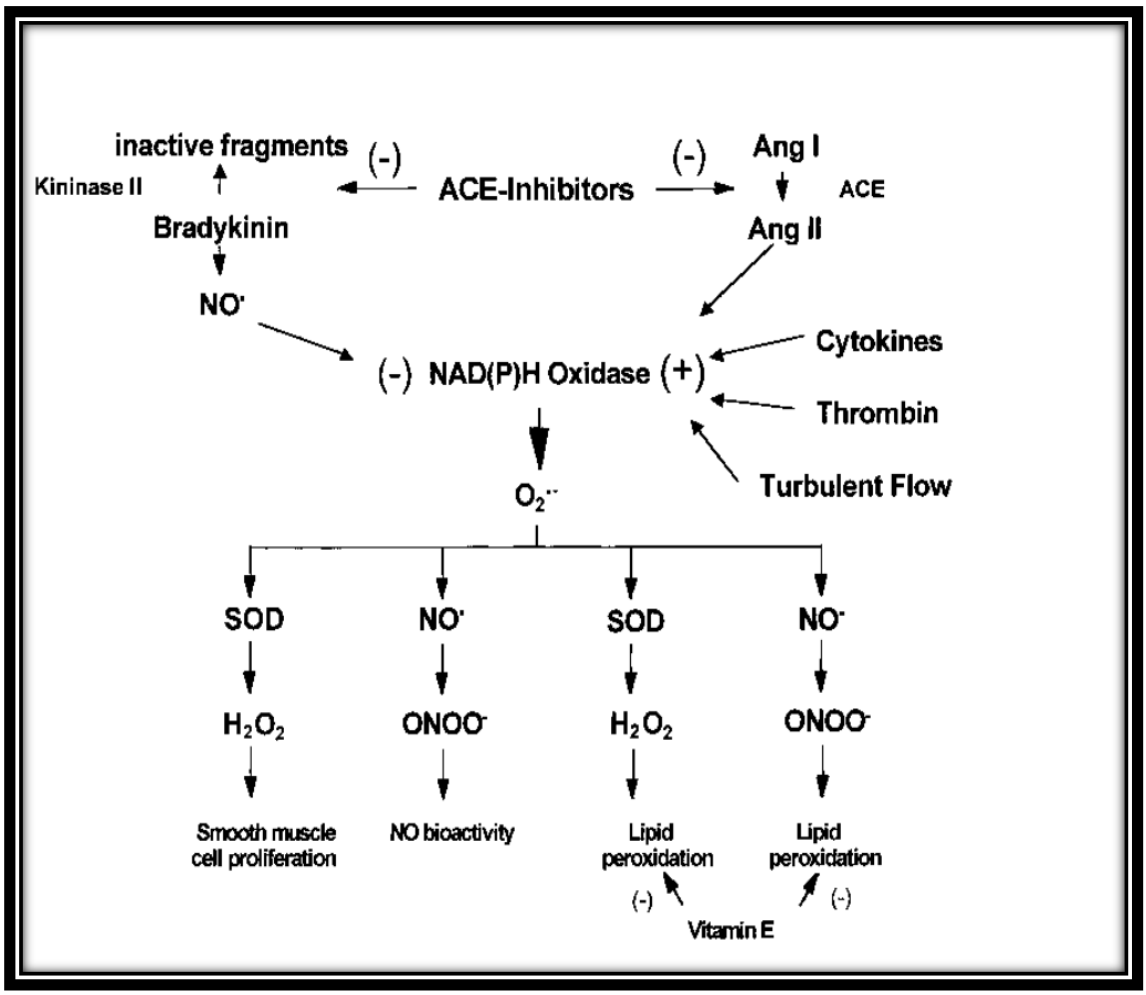
Richard et al[58].found that expression of bradykinin receptor & ACE inhibition on oxidative stress during anthracycline-induced cardiomyopathy in animal models.

ACE inhibitors have an antioxidant property which targets oxidative stress at its source level confirmed by the link between vascular NAD(P)H oxidase activity and ACE action. Enalapril inhibits NAD(P)H oxidase stimulation, by which it prevents renin-angiotensin system mediated superoxide flux. In hypertension and coronary artery disease superoxide reacts with NO, which further inhibit the NAD(P)H oxidase activity. NO bioactivity is improved by ACE Inhibition which in turn reduces the superoxide levels in the vascular wall. ACE inhibition also minimizes the lipid peroxidation via reduced peroxynitrite production.

Thus ACE inhibitors limit the smooth muscle proliferation, and carotid intimal thickening as confirmed by SECURE study due to its inhibition of H_2O_2. Because ACE inhibitors will minimize H_2O_2

production. H_2O_2-derived hydroxyl radical and hypochlorous acid (HOCl) formation also be decreased by ACE inhibitors. Finally, ACE inhibitors reduce the production of oxidants at the source level; issues of scavenging efficiency and compartmentalization are not a consideration.

Figure-17 Antioxidant activity of ACE Inhibitors



ROLE OF ACE INHIBITORS IN DOXORUBICIN INDUCED CARDIOTOXICITY[62,66,67]:-

Doxorubicin induced cardiotoxicity is mainly attributed due to oxidative stress to the myocardium and later up regulation of ACE activity which in turn progress to Angiotensin II mediated cardiac dysfunction.

Since ACE Inhibitors has free radical scavenging activity by inhibiting NADPH Oxidase mediated oxidative stress to the endothelium and myocytes and also inhibit the ACE over activity in anthracyclines induced cardiomyopathy. So it may ameliorate doxorubicin injury to the heart because of its dual role in preventing cardiotoxicity.

Sacco et al[57]. have studied that role of zofenopril an ACEI in reducing cardiac injury due to the long term doxorubicin administration in rats. After oral administration of zofenopril which significantly reduced adriamycin-induced cardiotoxicity without reduce the antineoplastic activity of Adriamycin.

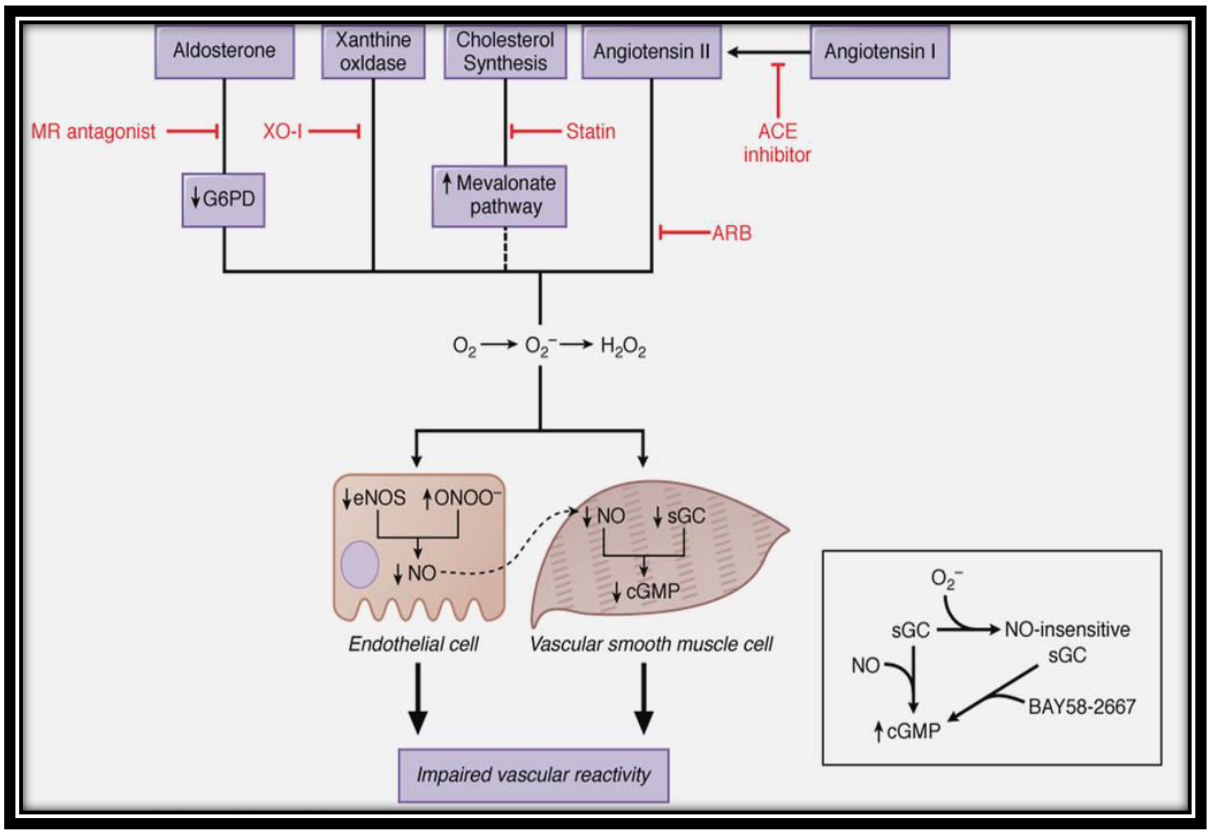
Abd el-aziz et al.[63] demonstrated the anti oxidative potential of both enalapril & captopril in daunorubicin -induced oxidative toxicity in rats. This cardio protective effect may be mediated by the limitation of free radicals and the amelioration of oxidative stress. Iqbal et al[64].demonstrates

the protective effects of telmisartan against acute adriamycin-induced cardiotoxicity in rats.

Recent trend in CHF management[61]

An elevated level of oxidant and other forms of inflammatory stress observed in patients with CHF may impair vascular reactivity by disruption of normal vasodilator cell signaling pathways. Vascular dysfunction is associated with decreased exercise tolerance and a poorer clinical outcome. This process is in part mediated by increased levels of reactive oxygen species, decreased endogenous levels of antioxidant enzymes, and decreased levels of bioavailable NO. Preserving normal vascular reactivity is a target of evolving priority in the treatment of patients with chronic congestive heart failure. Increased levels of reactive oxygen species (ROS), including superoxide (O_2^-) and hydrogen peroxide (H_2O_2) that are generated in both endothelial cells (EC) and vascular smooth muscle cells (VSMC) impair key cell signaling pathways necessary for normal vascular function. Specifically, hyperaldosterone-induced decreased antioxidant enzyme activity in EC, such as glucose-6-phosphate dehydrogenase (G6PD), results in increased ROS formation. In EC, elevated levels of ROS impair vascular reactivity, in part, by decreasing endothelial nitric oxide synthase (eNOS) activity and increasing peroxynitrite (ONOO–) formation to decrease bioavailable nitric oxide (NO) levels.

Figure – 18 Role of ROS in the Pathophysiology of CHF & its prevention by different drugs including ACE Inhibitors



Mineralacorticoid (MR)-receptor antagonists, XO inhibitors (XO-I), HMG-coA–reductase inhibitors (statin), AT1 receptor blockers (ARBs), and angiotensin-converting enzyme (ACE) inhibitors block various cellular reactions associated with elevated levels of ROS and impaired vascular reactivity.

However, before a conclusive statement can be made on the potential usefulness of ACE Inhibitors as an adjunct to anthracyclines therapy, there is a need for further long-term chronic studies with ACE Inhibitors.

MATERIALS

&

METHODS

MATERIALS AND METHODS

The study was undertaken in breast cancer patients who undergone doxorubicin based chemotherapy, to find out the cardio protective effect of enalapril on doxorubicin induced cardiotoxicity by serial monitoring of Left Ventricular Ejection Fraction, Fractional Shortening by echocardiogram and serum Troponin I level.

STUDY CENTRE

The present study was carried out in the inpatients of Department Medical Oncology, Government Rajaji Hospital, Madurai after obtaining clearance from Institutional Ethical Committee, Government Rajaji Hospital, Madurai. Ref. Letter No. 4105/E4/3 /2013. [Annexure I].

COLLABORATING DEPARTMENTS

- Institute of Pharmacology, Madurai Medical College, Madurai.
- Department of Medical Oncology, Govt. Rajaji Hospital, Madurai.
- Department of Cardiology, Govt. Rajaji Hospital, Madurai.
- Department of Biochemistry, Madurai Medical college, Madurai.

STUDY DESIGN

It was a single center, open labeled, prospective, interventional study.

STUDY POPULATION

Breast cancer Patients admitted in Department of Medical Oncology, Government Rajaji Hospital, Madurai.

SAMPLE SIZE

60 adult female breast cancer patients on doxorubicin based chemotherapy, divided into two groups, each group comprising 30 patients after satisfying the inclusion and exclusion criteria.

STUDY PERIOD

This study was conducted from Feb 2013 to Aug 2014 for 19 months.

1. Literature collection : 3 months
2. Designing the study : 1 month
3. Case selection and follow-up : 12 months
4. Analysis & Interpretation : 2 months
5. Discussion : 1 month.

INCLUSION CRITERIA

1. Breast cancer patients of age between 20 – 65 yrs irrespective of tumour size, and stage of the disease undergoing doxorubicin based chemotherapeutic regimen.
2. Gender – Female.
3. Subjects willing for the study.

EXCLUSION CRITERIA

1. Age more than 65 years.
2. Male breast cancer patients.
3. Pregnant & lactating women
4. Patient with H/o hypersensitivity to Enalapril.
5. Known case of bilateral renal artery stenosis
6. Left Ventricular Ejection Fraction < 50 % by echocardiogram
7. Ongoing therapy with ACE inhibitors & angiotensin receptor blockers
8. Patients with hypertensive, ischemic and valvular heart disease and uncontrolled hypertension.
9. Patients with systolic blood pressure less than 90 mmHg

10. Patients with hepatic dysfunction - As evidenced by symptomatic liver disease or abnormality in liver function tests.
11. Patient with Chronic kidney disease (Creatinine Clearance ≤ 60 ml/min.)
12. Patient with elevated serum potassium level ≥ 5meq/ l.
13. Previous participation in a similar study.

INFORMED CONSENT

Subjects who were willing to participate were explained about the purpose, risks and benefits of the study verbally before enrolling to the study, in a language that was easy to understand by the subject. Written informed consent was obtained from all the patients, dated and signed by them. The participants were provided with the details of the investigator (Name, contact address, phone number), so that they may contact the investigator for any ailments, which arise due to the intervention.

DISCONTINUATION

1. Patients were given the freedom to quit the study at any time.

2. Subjects were withdrawn from the study, if they

- Develop any serious adverse effects to the drug.
- Underwent any surgery.
- Migrate to other place.
- Fail to comply with the proposed protocol.

METHODOLOGY

60 Breast cancer patients undergoing doxorubicin contain chemotherapy either post operatively as adjuvant therapy or preoperatively as neoadjuvant therapy, admitted in the department of medical oncology were included for the study. The investigations like complete haemogram, blood sugar, blood urea, serum Creatinine, serum Bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase(SGPT), Alkaline Phosphatase (ALP),Serum Proteins, Electrocardiogram(ECG) , serum electrolytes were done. A baseline assessment of plasma troponin I (TnI) level was measured by ELISA method. Left ventricular ejection fraction (LVEF) & Fractional Shortening (FS) were measured by echocardiogram. Patients with LVEF >50% were taken in to the study. Patients were allocated into two groups. Each group comprising 30 patients.

GROUP I (CONTROL - WITHOUT ENALAPRIL)

- Inj.Doxorubicin 50mg/m^2 intravenously once in 21 days for 6 cycles
- Inj.Cyclophosphamide 500mg/m^2 intravenously once in 21days for 6 cycles.
- Inj.5-Fluorouracil 500mg/m^2 intravenously once in 21 days for 6 cycles.

GROUP II (ENALAPRIL)

- Inj.Doxorubicin 50mg/m^2 intravenously once in 21 days for 6 cycles,
- Inj.Cyclophosphamide 500mg/m^2 intravenously once in 21 days for 6 cycles
- Inj.5-Fluorouracil 500mg/m^2 intravenously once in 21 days for 6 cycles.
- Tab. Enalapril 5 mg / once daily at bed time started after the 6^{th} cycle of chemotherapy schedule and slowly titrated upto 10 mg once daily and continued for 6 months.

Cardiac assessment was done by measuring Troponin I level at baseline, 24 hrs after first dose of chemotherapy and at the end of the chemotherapy

schedule (6th cycle). Cardiac function was also evaluated by serial measurement of LVEF and FS by echocardiogram at baseline, 3rd cycle,6th cycle, 6th month and 9th month of the study.

VISIT 1 [BASELINE]

Patients were reviewed after one week of screening and they underwent the Following:

- Obtained informed written consent.
- Recorded height, weight and calculated BSA.
- Performed physical examination.
- Measured vital signs - Pulse rate, Blood pressure as per method described above.
- Performed a detailed systemic examination
- Obtained blood samples for laboratory tests [Blood sugar, blood urea, serum Creatinine, Serum electrolytes, Liver function tests, Hb%, Troponin I] and urine sample for albumin, Sugar and deposits.
- Evaluated the left ventricular ejection & fractional shortening by ECHO.
- Dispensed the study drug to the test group for every 21 days for a period of 6 months after end of doxorubicin schedule and instructed

the patient to take enalapril and explained about the dose and time of intake.

- Informed about the possible adverse reactions to drug therapy and were given the investigator's details for reporting.
- The date of treatment initiation was documented and the patients were advised not to take any medication without the knowledge of the invigilator.
- The subjects were asked to bring the utilized drug strips during their next visit to ensure their compliance.

SUBSEQUENT VISITS

During subsequent visits,

- Patients were reviewed once in 21 days.
- The patients were enquired about the well being.
- Used strips of the study medication dispensed during previous visit were collected and checked.
- Recorded blood pressure and pulse and systemic examination was performed.
- The patients were assessed for any adverse events.
- The study drug was given for the 3 weeks.

- After the end of 6th month, all the clinical tests performed during the initial visit were repeated.

END OF THE STUDY

At the end of 9th month all investigations were done during the initial recruitment were repeated, and ECHO was also performed to assess the left ventricular ejection fraction and fractional shortening. The treatment efficacy was monitored with echocardiography assessment. The tolerability of the drugs was monitored by assessing adherence to treatment, serum potassium levels and any adverse reactions noticed either by the patients or by the invigilator.

STATISTICAL ANALYSIS

The data were analyzed with SPSS statistical software package (Version 16.0 SPSS Inc., Chicago, USA). The results were analyzed using Student's "independent" t test for between the groups and Student's "paired" t test for within the group. $P < 0.05$ will be considered as statistically significant.

RESULTS

RESULTS

Totally 60 female breast cancer patients, 30 in each group were recruited for the study. All the 60 patients were followed up to the end of the study. There was no drop out from the study.

Among the 60 female patients who completed the study, the age related distribution were as follows, In the enalapril group 16.6% patients were in the age group 30-39 years, 20% patients were in the age group 40-49 years, 30% patients were in the age group 50-59 years, 33.3% patients were in the age group > 60 years.

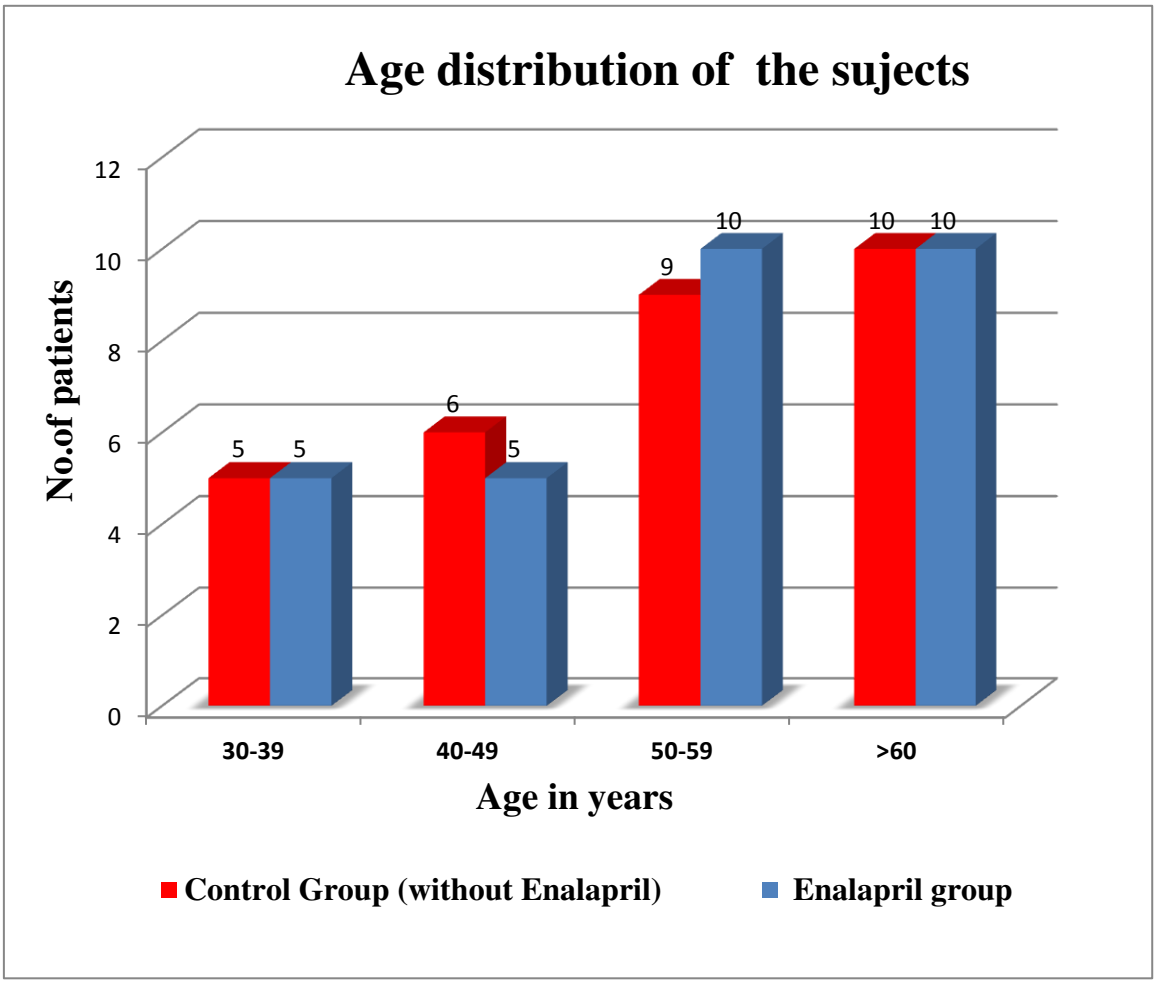
In control group (without Enalapril) 16.6% patients each were in the age group 30-39 and 40-49 years, 33.3% patients each were in the age group 50-59 and > 60years. The majority of patients belonged to > 60 years of age (33.3%) in both the groups.

The mean age of the patients in the control group was 53.33 ± 9.58 years. The mean age of the patients in enalapril group was 52.13 ± 9.80 years.

Table - 10 Age distribution of the participants

Age (years)	Number of patients	
	Control group	Enalapril group
30 – 39	5	5
40 – 49	5	6
50 – 59	10	9
>60	10	10
Total	**30**	**30**

Figure – 19 Age distribution of the participants



BASELINE PARAMETER

The following table shows the baseline characteristics of the patients in the both group included in the study.

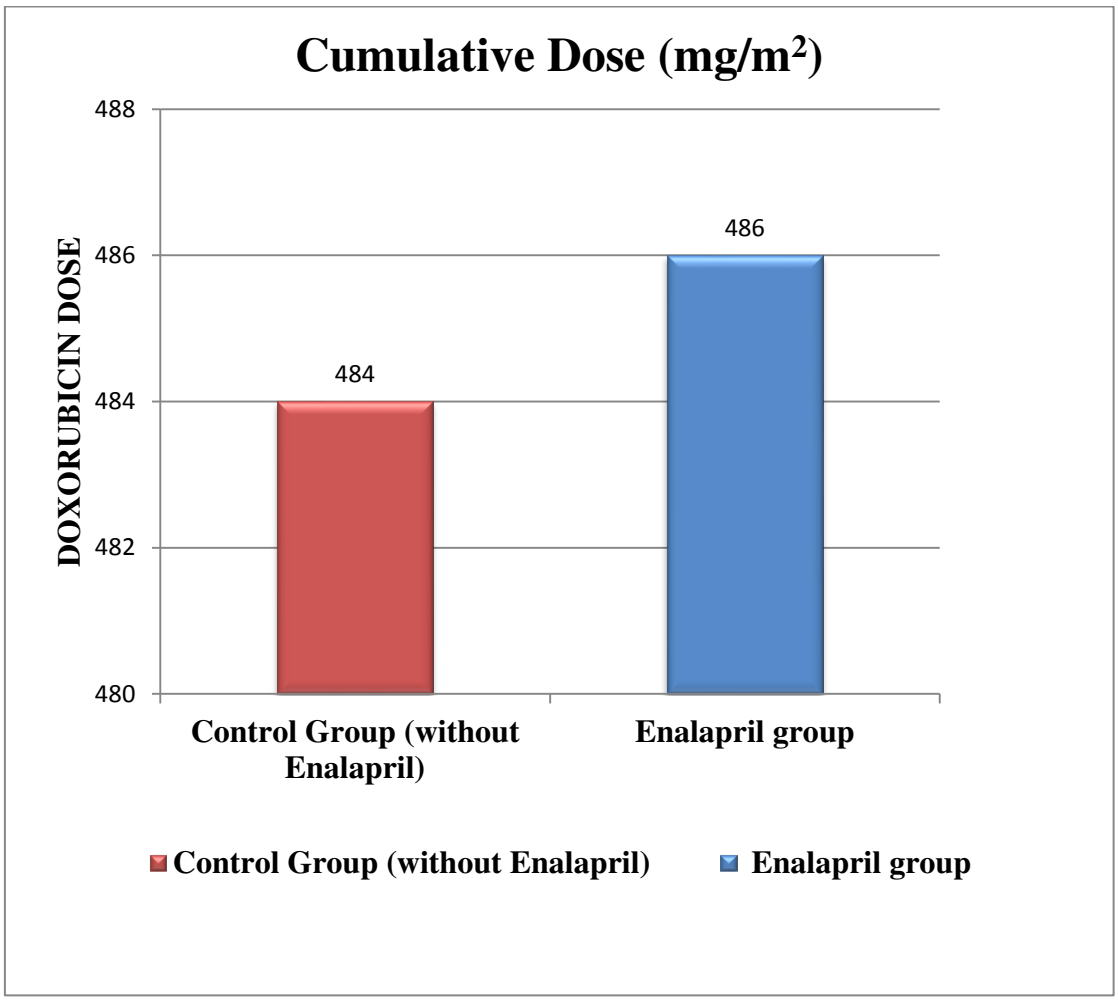
Table - 11 Baseline characteristics of the participants

PARAMETER	**Mean ± SD**	
	Control Group (without Enalapril)	**Enalapril Group**
Age (years)	53.33 ± 9.58	52.13 ± 9.8
BSA (m^2)	1.61 ± 0.12	1.62 ± 0.09
Number of chemotherapy cycles	6	6
Cumulative doses of doxorubicin	484 ± 36.93	486± 28.35
Baseline Heart rate	87.56 ± 10.32	89.02 ± 11.63
Baseline Systolic BP (mm Hg)	124.14 ± 13.52	128.06± 11.17
Baseline Diastolic BP (mm Hg)	81.23 ± 7.92	80.42 ± 9.07
Baseline Troponin I (ng/ml)	0.29 ± 0.14	0.25 ± 0.13
Baseline LVEF (%)	61.63 ± 2.98	61.45± 2.82
Baseline Fractional Shortening (%)	33.73 ± 2.22	34.03 ± 2.27

CUMULATIVE DOXORUBICIN DOSE

The mean cumulative doxorubicin dose of the study subjects at the end of the chemotherapeutic schedule (6th cycle) in Enalapril group and control group were 486 ± 28.35 and 484 ±36.93 respectively which is statistically not significant.

Figure – 20 Mean cumulative doxorubicin dose



TROPONIN I

Troponin I was used to categorize the high risk group as an early marker of myocardial injury. Plasma troponin I was measured using ELISA technique. After standardization the cut off value was determined as 1 ng/ml. The value > 1ng /ml was considered as early myocardial injury.

Average Troponin I value at baseline in Enalapril & control group was 0.25± 0.13 & 0.29± 0.14 ng/ml respectively. Within 24 Hrs of Doxorubicin infusion Troponin I level were elevated to 0.67± 0.60 & 0.63± 0.59 ng/ml in both the groups.

At the end of the chemotherapy schedule mean Troponin I value were 0.64±0.55 & 0.77±0.73 in both the groups respectively. 23.3% of patients in both the groups showed persistent elevation of Troponin I level and were more prone for cardiotoxicity and those subjects were considered as High risk groups.

Table – 12 Patients with Persistent Troponin I Elevation

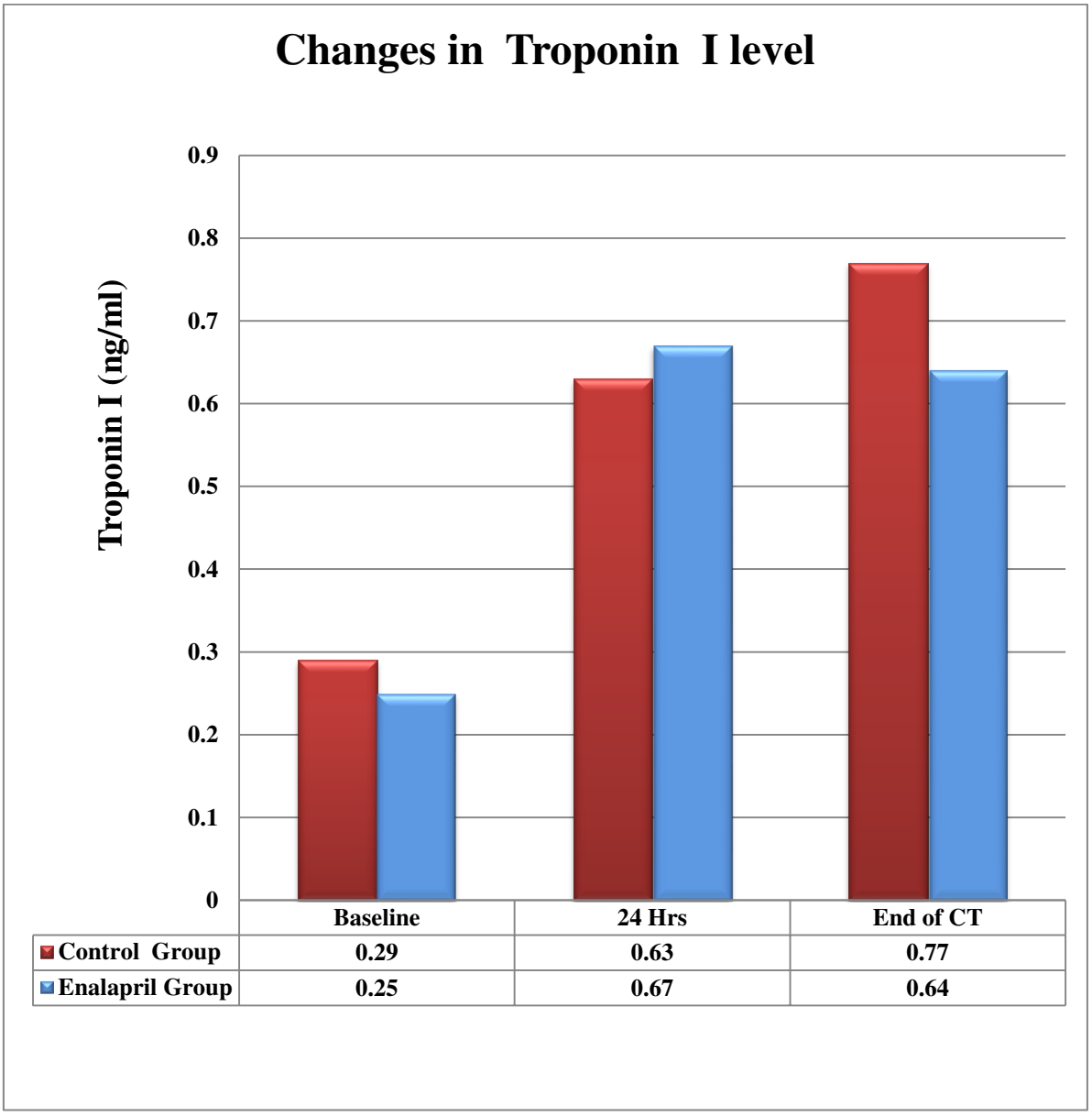
Timing of Troponin I	**No of patients with persistent Troponin I Elevation**	
	Control group	**Enalapril group**
24 hrs after doxorubicin	9 (30%)	8 (26.7%)
End of CT schedule	7 (23.3%)	7 (23.3%)

Table – 13 Plasma Troponin I values in both the groups

Timing of Tn I tested	**Troponin I value (ng/ml) (mean ±SD)**				
	Control Group	**Enalapril Group**	**T value**	**df**	**P value**
Baseline	0.29± 0.14	0.25± 0.13	-0.960	58	0.341
24hrs after first dose	0.63± 0.59	0.67± 0.60	0.257	58	0.798
6^{th} cycle	0.77±0.73	0.64±0.55	-0.724	58	0.472

*Significant if $P < 0.05$

Figure – 21 Plasma Troponin I values in both the groups



EJECTION FRACTION

Ejection Fraction is an important parameter to evaluate left ventricular function in doxorubicin induced cardiotoxicity. Hence Left ventricular function was monitored by serial measurement of Ejection Fraction with the help of Echocardiogram. The mean LVEF between groups were compared at baseline, 3rd, 6th cycle, 6th and 9th month of the study.

The mean LVEF at baseline , 3rd cycle and 6th cycle in both the groups were 61.47 ± 2.82 & 61.63 ± 3.02; 58.8± 3.16 & 58.87±3.50; 56.63 ± 4.10 & 56.83 ± 3.76 respectively. Comparison of LVEF between these groups were statistically not significant on "independent" t test. But within their respective groups they were statistically significant on students "paired" t test. The mean LVEF at 6th month in Enalapril treated group and control group were 60.20 ± 2.87 and 55.93 ± 4.33 respectively. Comparison of LVEF between these two groups showed that Enalapril treated group showed statistically significant improvement in LVEF than in control group ($P < 0.05$).

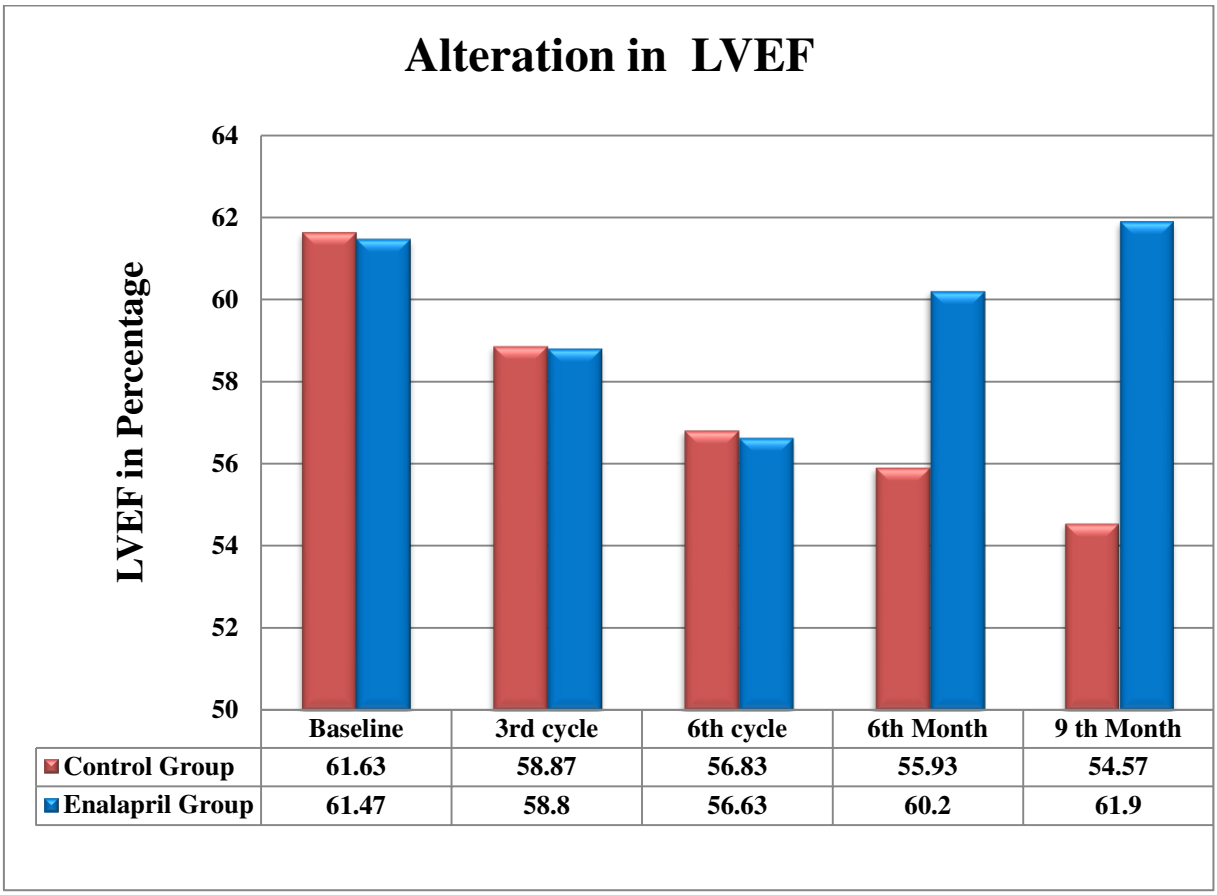
The mean LVEF at 9th month in Enalapril treated group and control group were 61.90 ±2.34 & 54.57 ± 5.86 respectively. Comparison of LVEF between these two groups showed that Enalapril treated group showed statistically significant improvement in LVEF than in control group ($P < 0.05$).

Table – 14 Changes in Left ventricular ejection fraction

Timing of ECHO	LVEF (%) MEAN ± SD				
	Control Group	Enalapril Group	tvalue	df	p value
Baseline	61.63 ± 3.02	61.47 ± 2.82	-0.221	58	0.619
3rd Cycle of CT	58.87 ± 3.50	58.80 ± 3.16	-0.077	58	0.819
6th Cycle of CT	56.83 ± 3.76	56.63 ± 4.10	-0.197	58	0.969
6th Month	55.93 ± 4.33	60.20 ± 2.87	4.493	58	0.023*
9th Month	54.57 ± 5.86	61.90 ±2.34	6.362	58	0.001*

*Significant P <0.001

Figure – 22 Changes in Left ventricular ejection fraction



FRACTIONAL SHORTENING

Fractional shortening is an another important parameter to evaluate left ventricular function in doxorubicin induced cardiotoxicity. Hence in this study, Left ventricular function was monitored by serial measurement of Fractional Shortening with the help of M mode Echocardiogram. The mean FS between groups were compared at baseline, 3^{rd} ,6^{th} cycle, 6^{th} and 9^{th} month of the study.

The mean FS at baseline, 3^{rd} cycle and 6^{th} cycle in both the groups were 34.03 ± 2.31 & 33.73± 2.56 ; 32.23 ± 2.10 & 31.90 ± 2.31; 30.77 ± 2.90 & 30.63 ± 2.51 respectively. When a change in FS was compared between these groups, they were not significant statistically on "independent" student's t test. But within their respective groups they were statistically significant on student's "paired" t test. The mean FS at 6^{th} month in Enalapril treated group and control group were 32.87 ± 2.53 and 29.60 ± 2.55 respectively. On comparing FS between these two groups Enalapril treated group showed significant improvement in FS than in control group ($P < 0.001$).

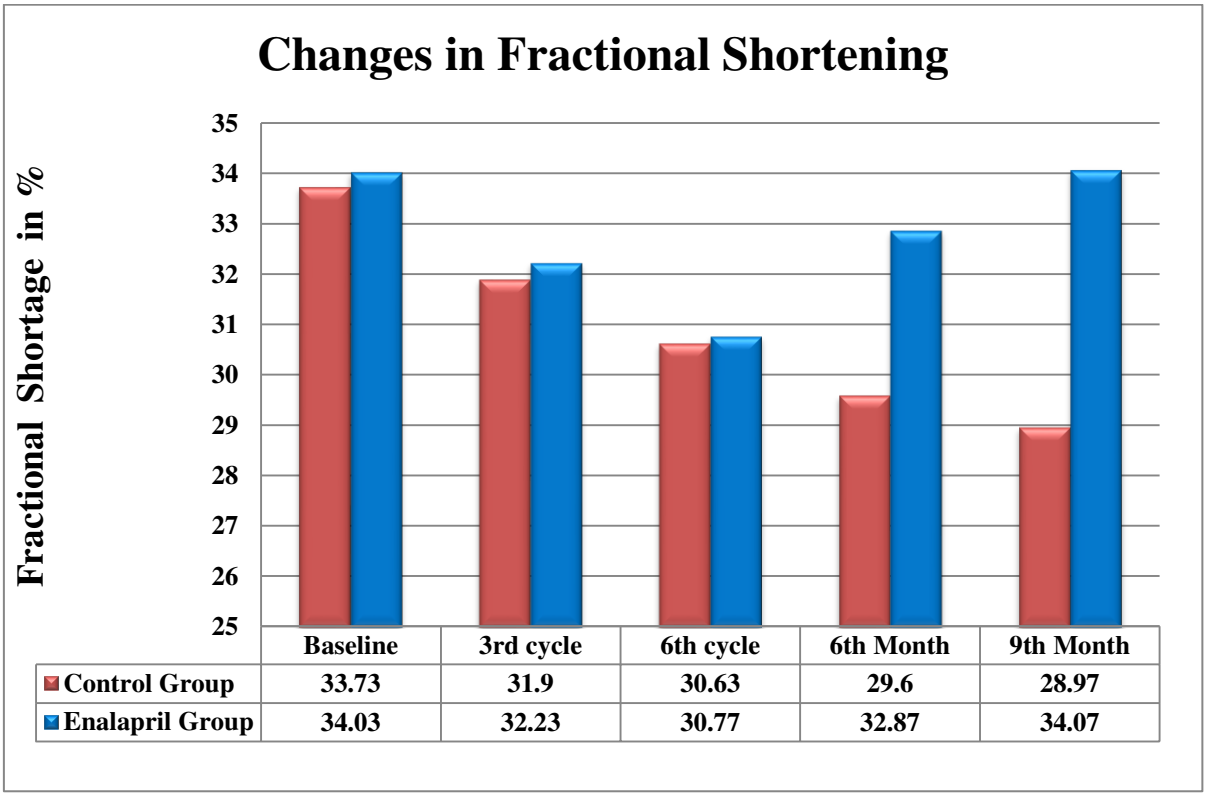
The mean FS at the end of study (9^{th} month), in Enalapril treated group and control group were 34.07 ±2.21 & 28.97 ± 3.47 respectively. When FS is compared between these two groups Enalapril treated group showed significant improvement in FS than in control group ($P < 0.001$).

Table – 15 Changes in the Fractional Shortening

Timing of ECHO	FS(%) MEAN ± SD				
	Control Group	Enalapril Group	t value	df	P value
Baseline	33.73 ± 2.26	34.03 ± 2.31	0.508	58	0.613
3rd Cycle of CT	31.90 ± 2.31	32.23 ± 2.10	0.585	58	0.561
6th Cycle of CT	30.63 ± 2.51	30.77± 2.90	0.199	58	0.843
6th Month	29.60 ± 2.55	32.87 ± 2.53	4.978	58	0.001*
9th Month	28.97 ± 3.47	34.07 ±2.21	6.776	58	0.001*

*Significant P <0.001

Figure –23 Changes in the Fractional Shortening



PERCENTAGE OF REDUCTION LVEF & FS OVER THE STUDY PERIOD

At the end of the chemotherapy schedule (6^{th} cycle), LVEF fell by 7.87% (56.63 ± 4.1) and 7.8% (56.83 ± 3.76)in both the groups from the baseline LVEF (61.47 ± 2.82 & 61.63 ± 3.02) respectively . After intervention with enalapril, at 6^{th} month the average LVEF was reduced by 2.06% (60.20± 2.87) and at the end of the study it was above the baseline (61.9±2.34). In control group, at 6^{th} month the average LVEF was reduced by 9.2% (55.93 ± 4.33) and at the end of the study it had fell to 11.4% (54.57 ± 5.86) from the baseline value.

At the end of the chemotherapy schedule (6^{th} cycle), FS fell by 9.4 % (30.77±2.9) and 9.2% (30.63 ± 2.51) in both the groups from the baseline FS (34.03±2.31 & 33.73±2.26) respectively. After intervention with enalapril, at 6^{th} month the average FS was reduced by 3.3% (32.87 ± 2.53) and at the end of the study it had reached above (34.07 ±2.21) the baseline value. In control group, at 6^{th} month the average FS was reduced by 12.2% (29.60 ± 2.55) and at the end of the study it had fell to 14.1 % (28.97 ± 3.47) from the baseline value.

Table – 16 Percentage of Reduction LVEF over the study period

Ejection Fraction	**Control group**		**Enalapril group**	
	Mean LVEF	**%of reduction**	**Mean LVEF**	**%of reduction**
Baseline	61.63± 3.0	0 %	61.47± 2.8	0 %
3rd cycle	58.87±3.5	↓4.5%	58.8 ± 3.16	↓4.3%
6th cycle	56.83± 3.7	↓7.8%	56.63 ± 4.1	↓7.8 %
6th month	55.93 ± 4.33	↓9.2%	60.20± 2.87	↓2%(↑5.9%)
9th month	54.57± 5.86	↓11.4%	61.90±2.34	0% (↑8.5%)

Table – 17 Percentage of reduction FS over the study period

Ejection Fraction	**Control group**		**Enalapril group**	
	Mean FS	**%of reduction**	**Mean FS**	**%of reduction**
Baseline	33.73±2.26	0 %	34.03±2.31	0 %
3rd cycle	31.9±2.31	↓5.4%	32.23±2.1	↓5.2%
6th cycle	30.63 ± 2.5	↓9.2%	30.77±2.9	↓9.4 %
6th month	29.6 ± 2.55	↓12.2%	32.87 ± 2.53	↓3.3%(↑6.3%)
9th month	28.97 ± 3.47	↓14.1%	34.07 ±2.21	0% (↑9.6%)

Figure -24 Percentage of Reduction LVEF over the study period

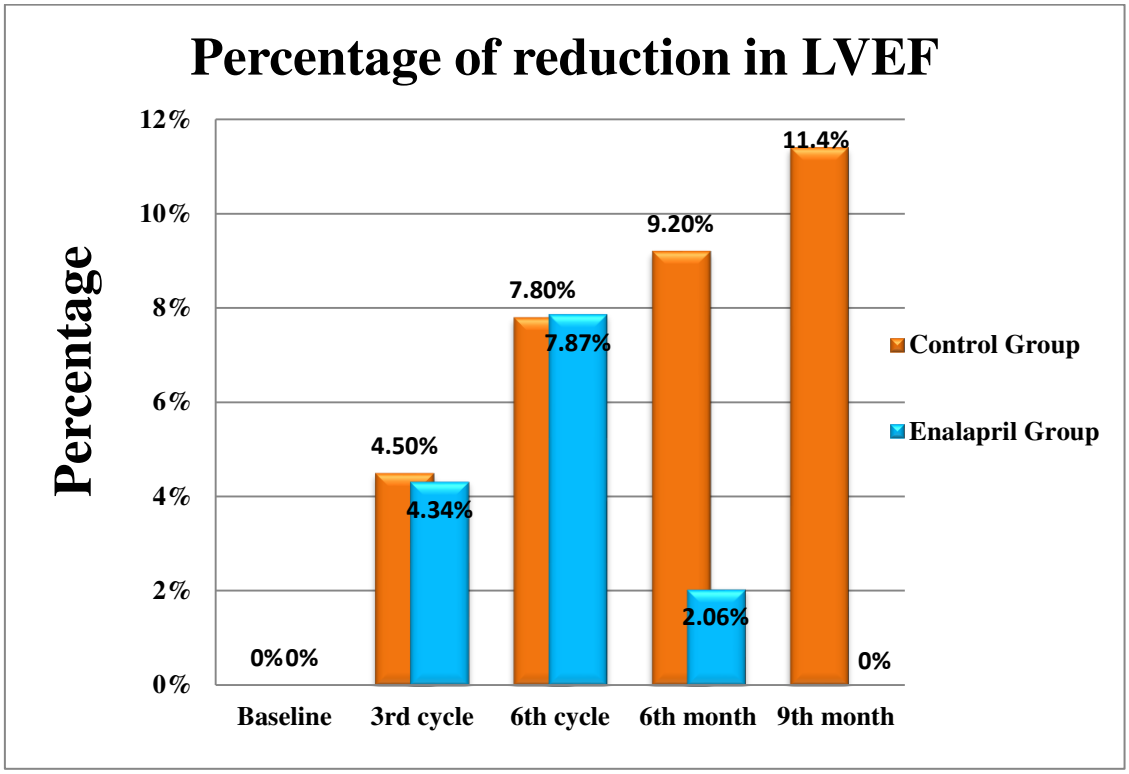
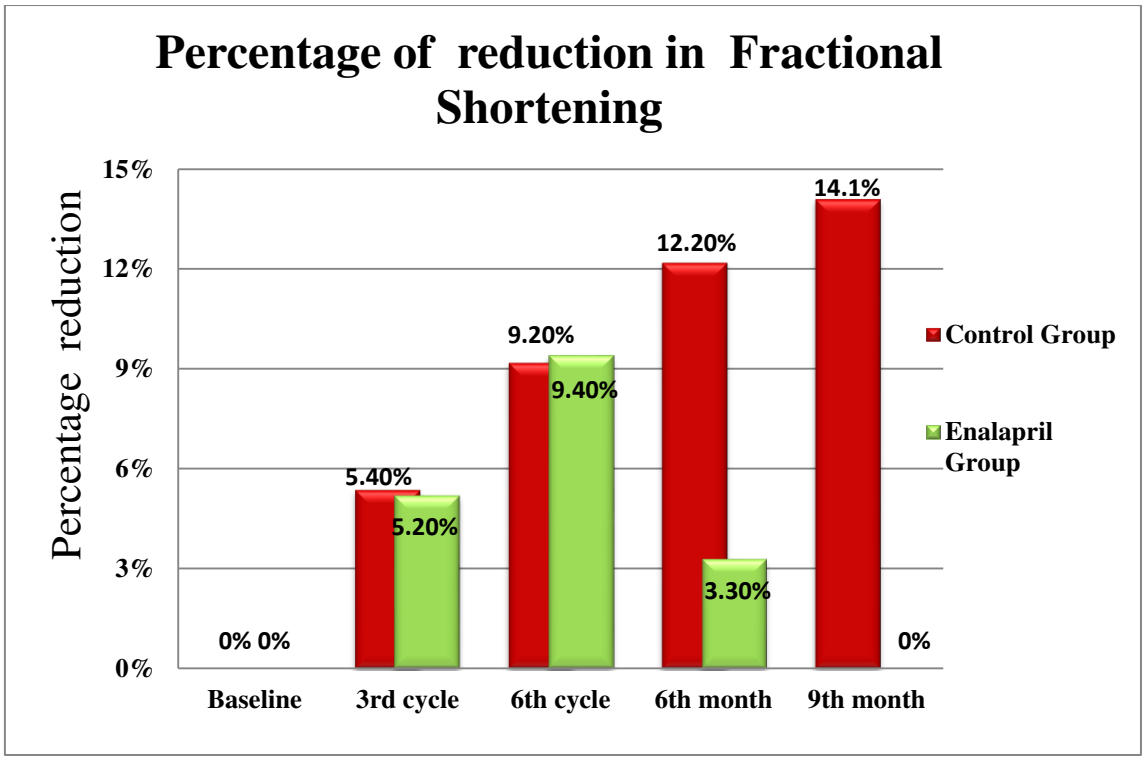


Figure – 25 Percentage of reduction FS over the study period



SUBCLINICAL TOXICITY

Tough clinical manifestations of doxorubicin induced cardiotoxicity manifest when its cumulative dose exceeds > 450 mg/m^2, sub clinical, asymptomatic cardiotoxicity increases by adding each dose of doxorubicin. Sub clinical toxicity defined as more than 10% reduction of LVEF and fractional shortening from its baseline value during serial echocardiogram evaluation.

In this study

Table – 18 No. of patients with LVEF < 50% & FS < 25%

Timing of ECHO	**No. of patients with LVEF < 50% & FS < 25%**	
	Control group	**Enalapril group**
End of CT	3 (10%)	2 (6.7 %)
End of study	6 (20%)	0 (0%)

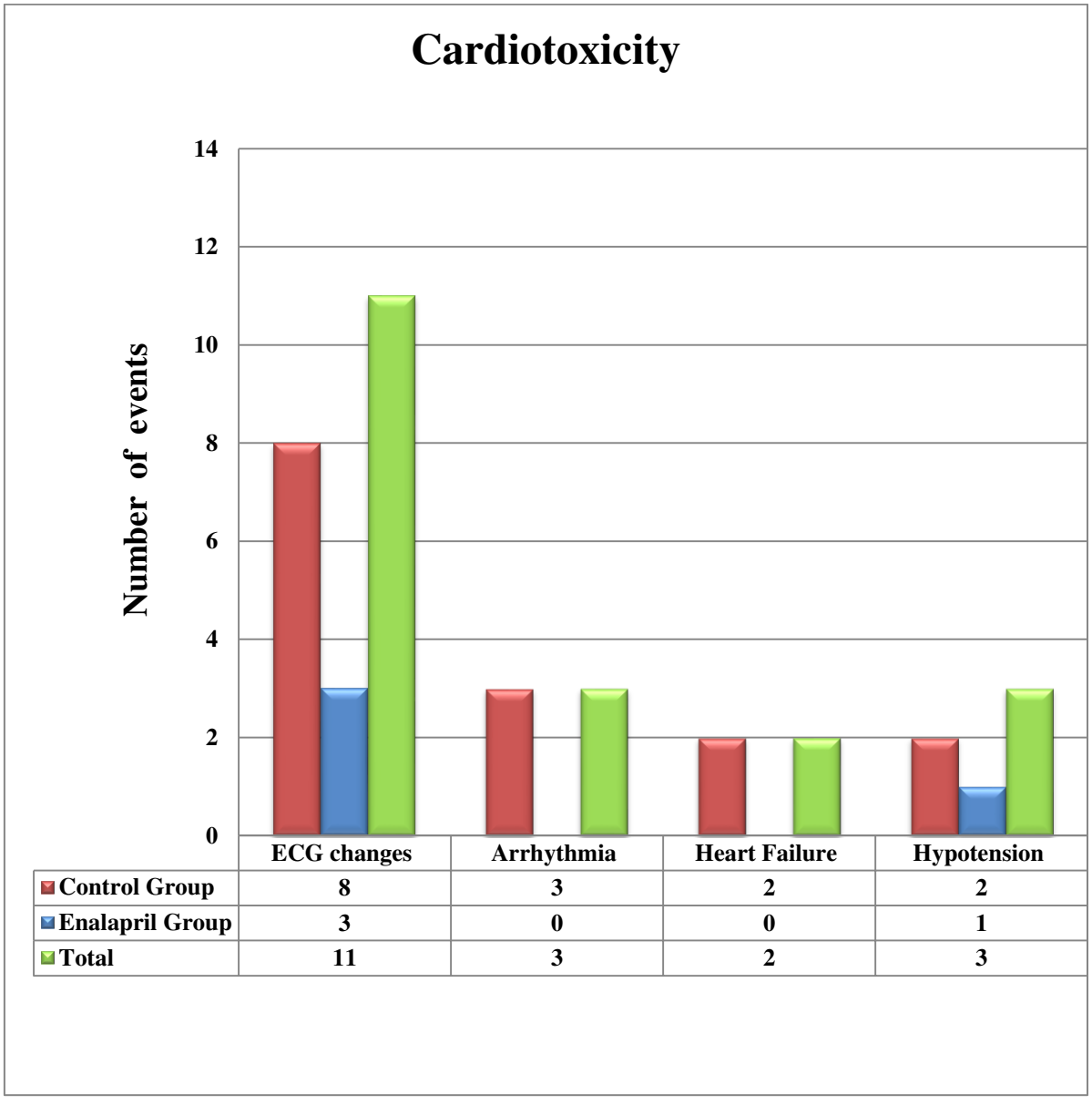
Table – 19 No. of patients with > 10 % reduction in LVEF & FS

Timing of ECHO	**No. of patients with > 10 % reduction in LVEF & FS**	
	Control group	**Enalapril group**
End of CT	7 (23.3%)	7 (23.3%)
End of study	11 (36.7%)	1 (3.3%)

Table – 20 Cardiac events during entire study period

EVENTS	Total	Control Group	Enalapril Group
ECG changes (Non specific)	11	8	3
Arrhythmia requiring treatment	3	3	0
Heart failure treated	2	2	0
Hypotension required Enalapril dose reduction / treatment	3	2	1
Hyperkalemia	Nil	Nil	Nil
Abnormal RFT	Nil	Nil	Nil
Abnormal LFT	Nil	Nil	Nil

Figure – 26 Cardiac events during entire study period



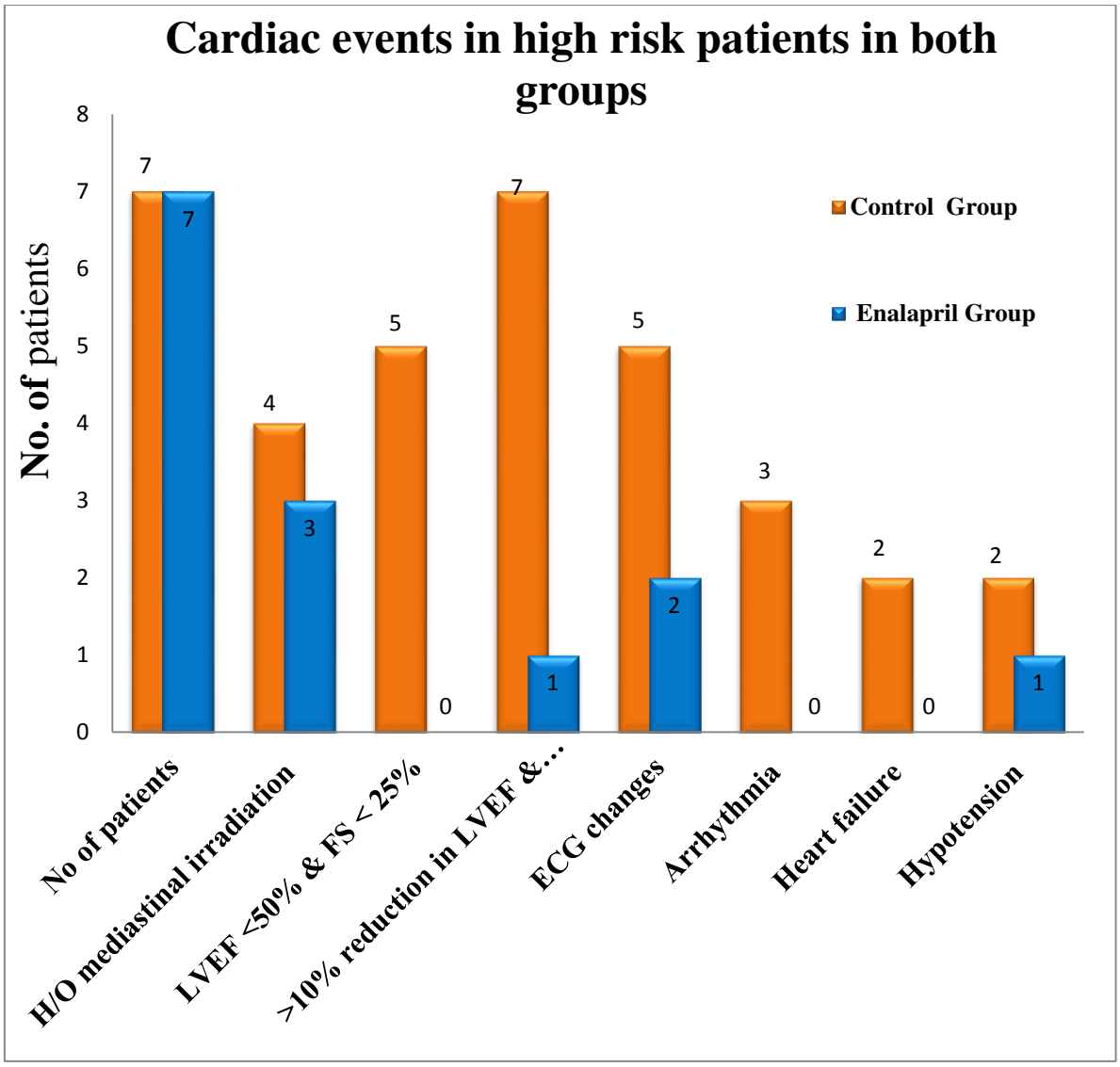
	ECG changes	Arrhythmia	Heart Failure	Hypotension
Control Group	8	3	2	2
Enalapril Group	3	0	0	1
Total	11	3	2	3

Table - 21

Cardiac events in patients with persistent Troponin I elevation

Cardiac events		**Control Group**	**Enalapril group**
No of patients		7 (23.3%)	7 (23.3%)
Patients with H/O Mediastinal irradiation		4(13.3%)	3(10%)
LVEF <50% & FS <25%	End of CT	3 (42.8%)	2 (28.5 %)
	End of study	5 (71.4%)	0 (0%)
> 10 % reduction in LVEF & FS		7 (100%)	1 (14.3%)
ECG changes (Non specific)		5 (71.4%)	2 (28.5 %)
Arrhythmia requiring treatment		3 (42.8%)	0 (0%)
Heart failure treated		2 (28.6%)	0 (0%)
Hypotension required treatment		2 (28.6 %)	1 (14.3%)

Figure – 27 Cardiac events in patients with persistent troponin I elevation

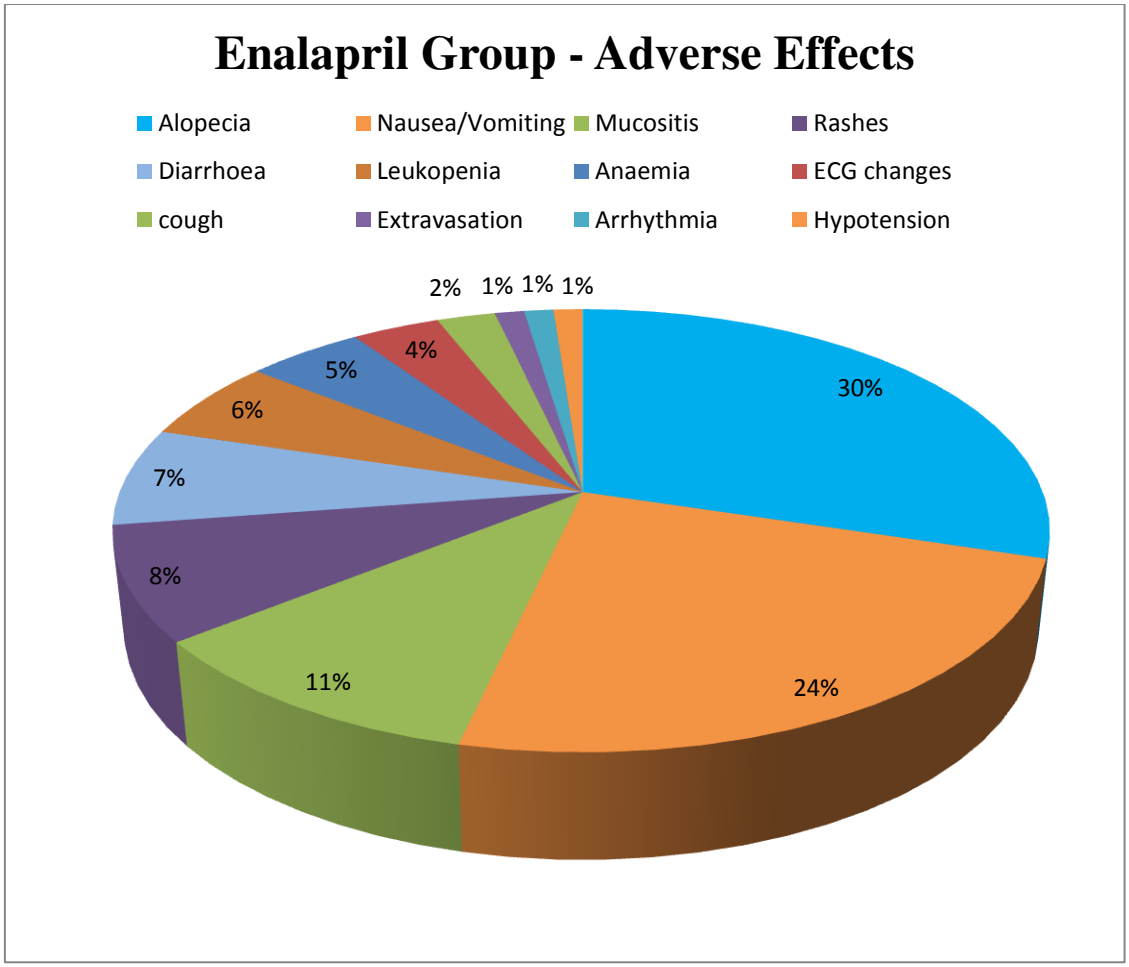


ADVERSE EFFECTS

The following adverse effects noted in enalapril treated group:-

Alopecia (30%), Nausea & Vomiting (24%), Mucosal ulcer (11%), Skin Rashes(8%), Diarrheoa(7%), Leucopenia(6%), Anaemia(5%), ECG changes(4%), Cough (2%), Extravasations, Arrhythmia and Hypotension each 1%.These are shown in pie diagram.

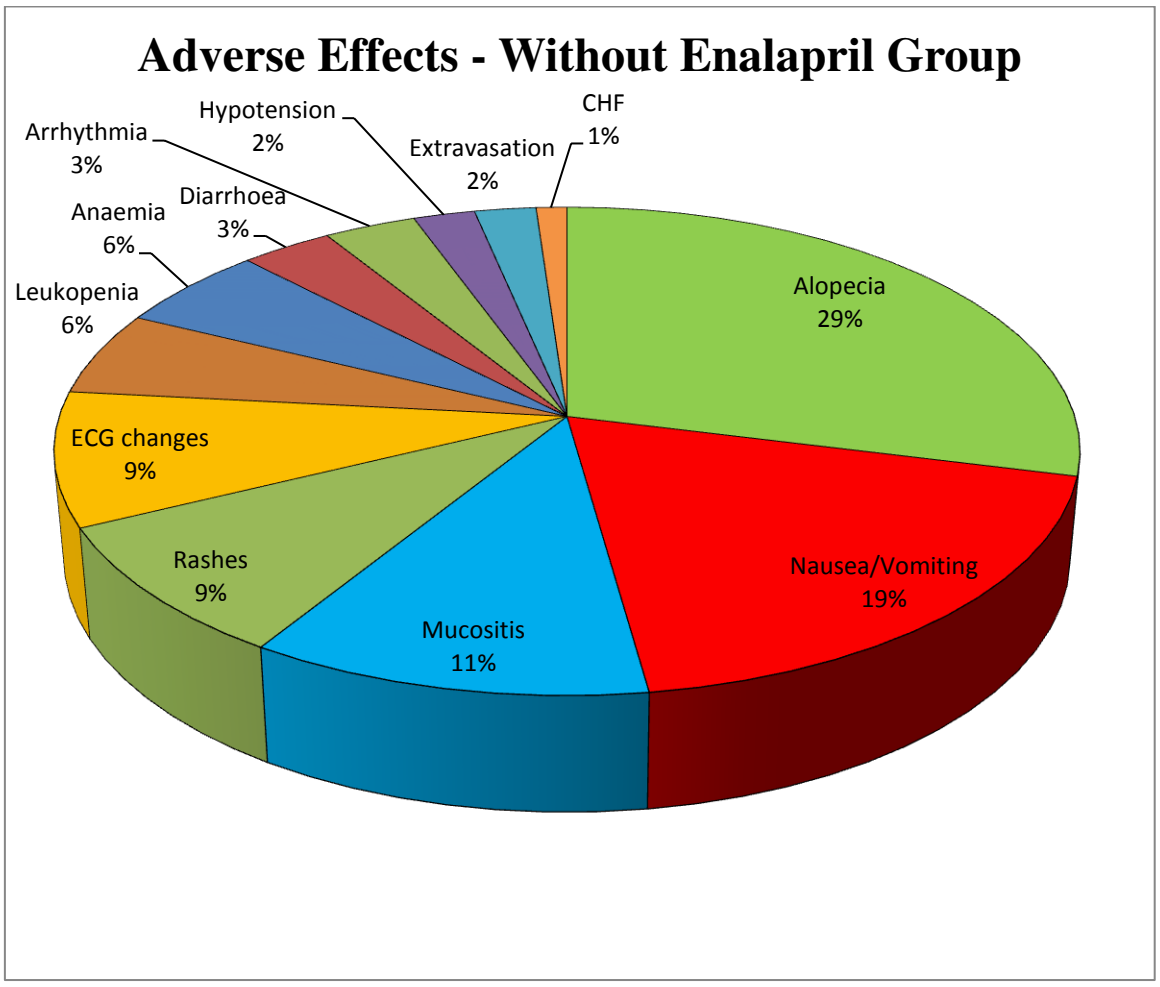
Figure – 28 Adverse effects noted in enalapril group



The following adverse effects noted in Control Group:-

Alopecia (29%),Nausea & Vomiting (19%),Mucosal ulcer (11%), Skin Rashes(9%), Diarrhea(3%), Leucopenia(6%), Anaemia(5%), ECG changes(9%), Extravasations(2%), Arrhythmia(3%), Hypotension(2%), Congestive Heart Failure(1%.). These are shown in pie diagram.

Figure – 29 Adverse effects noted in control group



DISCUSSION

DISCUSSION

Breast cancer is the most common cancer among females in developed countries like USA and UK. It is the second most common cancer of females after cervical cancer in developing countries like India. From the last decade onwards the incidence of breast cancer in urban populations increased dramatically. It is mainly due to western life style, changes in the food habits, sedentary life style, obesity, smoking habit and alcohol intake. Surgery is the primary modality of treatment in early breast cancers. For advanced stages adjuvant therapies like radiotherapy, chemotherapy and hormonal therapy are commonly used to prevent the metastasis and to prolong the disease free interval. Among chemotherapy anthracyclines based combination chemotherapy is commonly used to treat breast cancer because it is highly efficacious in reducing the tumor burden.

Doxorubicin is an antitumor antibiotic with wide spectrum of activity over the many neoplastic disorders including carcinoma of breast. Since 1970 its introduction as a cancer chemotherapeutic agent, it is one of the main component of various chemotherapeutic regimen in most of the solid cancers, hematological neoplasm's, lymphomas, sarcomas and carcinomas including breast cancer.

Drug induced cardiotoxicity is a rapidly evolving condition because of number of cancer survivors has been increased. Doxorubicin induced cardiotoxicity is mainly due to dose dependent cumulative toxicity

via free radical injury and oxidative stress to the cardiac myocytes. Doxorubicin can cause acute reversible cardiotoxicity and delayed irreversible cardiomyopathy years after doxorubicin therapy. Prevalence of doxorubicin induced cardiotoxicity is not known. Worldwide, the overall incidence of this cardiotoxicity is underestimated because of its delayed presentation. The onset of asymptomatic & subclinical[66] cardiotoxicity not only negatively impacts the cardiac outcome of breast cancer patients and also limits their therapeutic opportunities seriously.

Early prediction of cardiotoxicity by identifying high risk groups by measurement of cardiac biomarker like Troponin I soon after doxorubicin therapy, patients with elevated Troponin I are the greatest risk of development of cardiotoxicity in future eventhough asymptomatic at early stages. So it is mandatory to evaluate cardiac function of the patients undergoing doxorubicin therapy by means of serial measurement of left ventricular ejection fraction and fractional shortening by echocardiogram before, during and after doxorubicin therapy periodically and early intervention will prevent or delay the development of chronic cardiotoxicity. By prophylactic administration of Enalapril[67], an Angiotensin Converting Enzyme Inhibitor in high risk groups minimize the cardiovascular morbidity and mortality in different clinical settings including doxorubicin induced cardiotoxicity[50,55,62] by inhibiting Angiotensin II mediated oxidative stress to the cardiomyocyte, cardiac

remodeling and cardiac hypertrophy .

With this background the present study was undertaken to find out the effect of doxorubicin on cardiovascular system and to evaluate the cardio protective effect of enalapril on doxorubicin induced cardiotoxicity in a tertiary care center in south Indian population.

This study was undertaken in 60 female patients who were diagnosed as breast cancer, irrespective of the size and stage of the cancer were recruited and allocated into 30 in each group as per the protocol. Baseline investigations like complete haemogram, blood sugar, blood urea, serum Creatinine, serum Bilirubin, serum electrolytes were done. Cardiac function was assessed by ECG, plasma troponin I level. Patients with LVEF >50% & FS >25% were included for the study. Both the groups received six cycles of chemotherapy containing doxorubicin, cyclophosphamide & 5-Fluorouracil.

In the present study the mean age of participants in enalapril and without enalapril group respectively were 53.3±9.5 & 52.1±9.8 years. Majority of patients belonged to post menopausal age group between 50 – 65 years in both the groups.

Cardinal et al. demonstrated that TnI elevation soon after doxorubicin chemotherapy predicts cardiotoxicity and its poor cardiological outcome

earlier, with more risk observed in patients having persistent TnI elevation[7].

In the present study TnI measured at baseline, 24hrs after first dose of doxorubicin and at the end of chemotherapy schedule (6^{th} cycle) used to predict early myocardial injury. 26.7% & 30% of subjects in both the groups were showed elevated TnI after 24 hrs. 23.3% of the participants in both the groups showed persistent elevation of TnI at the end of chemotherapy schedule were considered as high risk patients. These findings are similar with cardinal et al[11] study.

After the completion of six cycles of chemotherapy first group consists of 30 patients were started Enalapril 5mg/day orally at bedtime and the dose gradually increased up to 10 mg per day continuously for 6 months. Remaining 30 patients did not receive enalapril and both group of patients were observed for 9 months.

Doxorubicin induced subclinical and clinical cardiotoxicity was monitored by ECG, Ejection fraction & Fractional shortening by echocardiogram at 6^{th} and end of the study (9^{th} month) in all these participants. Left ventricular function was monitored by serial measurement of LVEF & FS by echocardiogram. In this study both these parameters were monitored at baseline, end of chemotherapy schedule, 6^{th} & 9^{th} month of the study.

Normal ejection fraction value is between 50-70%.Normal fractional shortening value is between 25-45%. Symptoms of Left ventricular dysfunction occur when LVEF < 45% & FS < 25%.

In the present study, patients treated with enalapril showed statistically significant improvement in LVEF & FS as compared with patients without enalapril. When compare LVEF & FS between these two groups, mean LVEF & FS were gradually decreased in control group at the end of doxorubicin schedule, 6^{th} month & 9^{th} month of the study from the baseline value which is statistically significant ($p < 0.001$). These findings are similar with cardinal et al[11] study.

In enalapril treated group mean LVEF & FS were gradually increased at the 6^{th} month and 9^{th} month of the study from the mean LVEF & FS at the end of chemotherapy cycle which is statistically significant ($p < 0.001$).At the end of the study mean LVEF is attained the baseline value in enalapril treated patients.

In control group both LVEF & FS was gradually reduced to 7.8% & 9.2%; 9.2% & 12.2%; 11.4% & 14.1% respectively at the end of chemotherapy,6^{th} & 9^{th} month of the study from the baseline value. But in enalapril treated group both LVEF & FS was gradually increased to 5.9% & 6.3% at 6^{th} month, 8.5%&9.6% at 9^{th} month respectively compared to the values at the end of chemotherapy (6^{th} cycle) . Both LVEF & FS reaches the baseline value at the end of 9^{th} month.

Asymptomatic, subclinical cardiotoxicity [66,68,] is defined as more than 10% reduction in LVEF & FS from the baseline value and those LVEF & FS values are >50% & >25% respectively. The patients with subclinical toxicity are more prone to develop congestive cardiac failure and cardiomyopathy in future.

In this study 36.1% patient & 3.3% patients in control and enalapril treated groups showed sub clinical cardiotoxicity by means of more than 10% decrease in both LVEF & FS from the baseline value. These findings are comparable with khattry et al[68] study findings.

At the end of 9th month 20% of patients in control group showed both LVEF & FS less than 50% & 25%. But in enalapril treated group all the 30 patient's LVEF & FS were above the normal value.

ECG changes like sinus tachycardia, prolonged PR interval, T wave inversion were seen in 10% & 26% of patients in enalapril treated and control groups respectively. All these changes were reverting to normal in both these groups. Arrhythmias developed in 10% of patients in control group and were treated successfully. Symptomatic heart failure occurred in 2 persons (6.6%) in control group and was treated. No one developed arrhythmia & CHF in enalapril treated group. Hypotension was developed in one patient in each group, enalapril dose was reduced from 10mg to 5mg in enalapril treated patient and also treated with IV fluids.

Cardiac events in high risk group (elevated TnI):-

At the end of 9th month ,5 patients (71.4%) showed LVEF <50% & FS < 25% out of 7 patients showed persistent TnI elevation in control group, none in enalapril treated group. Sub clinical cardiotoxicity were found in 100% & 14.3% respectively in control & enalapril treated high risk group category. CHF, Arrhythmia, Hypotension were developed in 28.6%,42.8% &14.3% respectively in high risk control group,0%,0% & 14.3% in enalapril treated high risk group. These findings are similar with cardinal et al.[11] study. Most of these cardiotoxicity occurred in age more than 55 yrs, cumulative dose more than 450mg/m^2, and H/O mediastinal irradiation.

Common adverse effects noted in both groups were alopecia, nausea and vomiting, mucositis, diarrhea and rashes.

The role of cardiac Renin Angiotensin System in the formation of doxorubicin-induced cardiotoxicity is documented in experimental models. Cardiac ACE activity was elevated after doxorubicin therapy compared with control subjects in those models. In an animal model lisinopril given after doxorubicin therapy significantly decrease the cardiac ACE activity. Lisinopril also improved the cardiac function, mortality, reduced the ventricular remodeling. Collagen accumulation and fibrosis was inhibited in the cardiac muscle in temocapril treated rats. These findings suggest that activation of ACE plays an important role in the development of cardiotoxicity. So ACEIs beneficial effects in

doxorubicin- treated animals depend on its inhibition of cardiac ACE.

Enalapril had been shown to have antioxidant property by its free radicals scavenging activity. An oxidative stress is a possible mechanism in the formation of doxorubicin induced cardiomyopathy. Hence both Sulfhydryl- and non–Sulfhydryl-containing ACEIs may be effective in the prevention of doxorubicin -induced cardiotoxicity because of its free radical scavenging potential on cardiomyocyte.

Cardiac tissue RAS stimulation and oxidative stress to the myocardium may be involved in the progression of doxorubicin -induced cardiac injury. Hence by limiting these mechanisms with enalapril, may be useful to prevent the development of cardiac dysfunction.

The present study results confirm the prognostic role of TnI as an early marker to find out the high-risk patients. Prophylactic enalapril administration has been showed to preserve the left ventricular function & improved cardiological outcome. Neither fractional shortening nor LVEF altered during the treatment period in the enalapril treated group, and lower incidence of cardiac events was observed than in untreated patients. The benefits of ACEIs have been defined in hypertension, acute myocardial infarction, and asymptomatic left ventricular dysfunction and in chronic heart failure. Use of enalapril has been proved to be beneficial in prolonging survival and in preventing further deterioration of cardiac function in anthracycline- induced cardiotoxicity.

Untreated patients having a persistent TnI increase after the end of doxorubicin therapy have progressive LVEF reduction than in patients with normal TnI level. Benefit of enalapril treatment was present in both subgroups, but patients with a persistent TnI elevation may particularly benefit from enalapril therapy. Enalapril was very well tolerated in all the patients because of very slow and careful titration may have contributed to this result.

Different strategies have been implicated to reduce or to prevent cardiac toxicity. Like close monitoring of cardiac function, modification in doxorubicin schedule administration, minimize the doxorubicin cumulative dose, doxorubicin analogue usage and use of cardioprotectants like antioxidant agents, dexrazoxane have been followed. However, each of these approaches has its own limitations such as the possible compromising of antitumor efficacy, poor predictive value and high costs.

Study Limitations

Although this trial was designed as a prospective study, the lack of placebo administration, open-label follow-up were potential limitations of this study. However, the percentage of patients treated with doxorubicin schedule, and the doxorubicin cumulative doses were well balanced in both the groups. Hence a different risk of cardiotoxicity in both the group is unlikely.

SUMMARY

&

CONCLUSION

SUMMARY AND CONCLUSION

Breast cancer is the second most common cancer of females in India next to cervical cancer. It is managed by multi model approach like surgery, radiotherapy, chemotherapy & hormonal therapy. Chemotherapy is used to prevent tumor progression & to prevent tumor recurrence.

Doxorubicin is the most active agent for the treatment of carcinoma breast. However, its use is limited by cumulative, dose related cardiotoxicity. Cardiotoxicity causes in a irreversible loss of cardiomyocyte and a progressive deterioration of left ventricular function following subsequent dose of doxorubicin therapy. Initially, it causes subclinical damage to the heart. However later it impairs cardiac function and result in serious cardiac injury leading to chronic heart failure and cardiomyopathy which is irreversible.

The mechanism of anthracyclines induced cardiotoxicity is not well understood till now. But the most accepted one is free radical injury mediated lipid peroxidation and oxidative stress to the myocardium which induces the apoptosis of cardiomyocyte and results in cardiac dysfunction. Some preclinical studies found that increased level of Angiotensin converting enzyme (ACE) during doxorubicin induced myocardial damage which stimulates angiotensinogen II mediated NADPH oxidative stress results in cardiac remodeling and heart failure. From this Renin Angiotensin

System (RAS) may also take part in the doxorubicin induced myocardial injury. So early detection and treatment of cardiotoxicity can reduce its cardiovascular morbidity and mortality which will occur later. Various strategies were used to reduce doxorubicin induced cardiotoxicity including alteration of doxorubicin dosing schedule, using liposomal formulations, reducing cumulative doxorubicin dose less than 450mg/m^2 , using anthracyclines analogues like epirubicin ,using non anthracycline chemotherapeutic regimen.

Though dexrazoxane – an iron chelating agent is used to treat doxorubicin induced cardiotoxicity after cumulative dose of 300mg/m^2,its long term protective effect on prevention of delayed cardiotoxicity is uncertain. Few trials found that it also reduce the anti tumor effect of anthracyclines if we used concurrently and its long term use may increase the incidence of secondary leukemia. So it should be used with caution because of lower response rate and the additional costs of the treatment.

Angiotensin converting enzyme inhibitors (ACEIs) are commonly used to treat hypertension, coronary artery disease, congestive heart failure. It is one among few group of drugs used to reduce the cardiovascular morbidity and mortality in various clinical settings. Its mortality reducing benefit is mainly due to its prevention of Angiotensin II mediated NADPH oxidase induced cardiovascular changes and improves cardiac left ventricular

function. So most of the ACEIs including Enalapril has free radical scavenging activity by preventing NADPH oxiadase induced lipid peroxidation in cardiomyocyte. So Enalapril was used in this study to limit the doxorubicin induced cardiotoxicity since it has antioxidant property and also ameliorates RAS mediated cardiovascular dysfunction.

Cardiac Troponin I is commonly used to identify myocardial injury at the earliest and used to pick up the high risk patients who are receiving conventional doxorubicin therapy. In this study 24% of patients in each group showed persistent elevation of Tn I at the end of doxorubicin based chemotherapy schedule. Most of the cardiac events occurred in this high risk patients compared to patients with transient TnI elevation and those showed normal TnI value.

In the present study shows the prognostic role of TnI and have preserved left ventricular function and an improved cardiological outcome when prophylactic therapy with enalapril is carried out.

From this we can conclude enalapril has therapeutic value in doxorubicin induced cardiotoxicity. Early identification of asymptomatic subclinical cardiotoxicity by monitoring LVEF & FS with the help of echocardiogram, and prophylactic administration of Enalapril can improves left ventricular function and to delay the progression of delayed irreversible cardiotoxicity in patients exposed to the doxorubicin

for life saving anti cancer therapy.

Further long term, large population based multicenter studies need to be carry out to confirm the enalapril role in prevention of doxorubicin induced cardiotoxicity.

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ANNEXURES

PROFORMA

Name:- Age:-

Sex :- IP No:-

Occupation:-

Address:- Phone no:-

H/o presenting illness :-

Swelling – Onset,Duration

Pain -

Discharge

Axillary Swellings

H/O Metastasis

Past history :-

Any H/o Comorbid Illness:-

Family H/O :-

Menstrual history :-

Personal History :-

Treatment History :-

Drug History:-

General examination

Height : Weight : BSA:-

O/E

Conscious

Oriented

Pallor

Cyanosis

Clubbing

Pedal edema

Generalized lymphadenopathy

VITALS

Pulse : B.P :

R.R : Temp :

Examination of breast & axilla :

Nipple & Areola – Discharge, Ulcer

Skin Over Breast - Dimpling,Retraction

Lump – Site , Size, Margins & Fixity

Lymphnodes – Number, Fixity

Systemic - Liver Spine/Bone

CVS-

RS-

P/A-

CNS-

BASELINE INVESTIGATIONS

FNAC :-

Mammogram :-

Chest radiograph:-

Receptor status:-

ECG:-

Complete hemogram:-

Parameter	Baseline	3rd month	6th month	9th month
LFT				
Blood urea				
Serum creatinine				
Serum Potassium				
Blood sugar				
Urine albumin, sugar, specific gravity,				

CARDIAC PROFILE.

Parameter	Baseline	After 24 hrs	6th Cycle
Troponin **I** Level			

Echocardiogram	Baseline	3rd Cycle	6th Cycle	6th Month	9th Month
LVEF					
FS					

ANY ADVERSE EFFECT

INFORMED CONSENT FORM IN ENGLISH

Full name of the patient(in capital letters): _____

Address:_____

_____Date of Birth:_____

Patient no:_____ Sex :_____ I freely agree to participate in the above – mentioned clinical study.

My doctor_____ informed me in a personal counseling interview about the study drug, possible side effects and risks, the nature, objective and significance of this clinical study and my responsibilities resulting thereof. In addition, I read and understood the contents of the Patient Information Sheet and Informed Consent Form. The doctor answered all questions in an adequate and comprehensible manner. I had sufficient time to decide on my participation in this clinical study.

I will follow the instruction of my doctor, which are essential for the performance of this clinical study. I have the right to withdraw from the study at any time without giving any reason and without any disadvantage for me.I confirm that I have not participated in this study and I have not taken part in another study within the last 30 days prior to the start of the study.

I received one original of the Patient Information Sheet together with the signed Information Consent Form.

_____

(Place, Date & Signature of the Patient)

_____

(Place, Date & Signature of the Doctor)

PATIENT INFORMATION SHEET

Who can be contacted for further questions?

For further questions regarding this clinical study or your rights as patient and participant in the study, please contact your doctor who will always be ready to provide you the necessary information.

If you have experienced any health related problems as well as in case of hospitalization please contact your doctor.

Name and Address of the Contact Person:

_____

_____

Phone number: _____

Please take a copy of this information sheet home with you.

INFORMED WRITTEN CONSENT FORM IN TAMIL

நோயாளிகளின் தகவல் மற்றும் ஒப்புதல் படிவம்

மார்பக புற்றுநோயாளிகளுக்கு டாக்சோரூபிசின்

சிகிச்சையினால் இருதயத்தில் ஏற்படும் பாதிப்புகளை

எனலாபிரில் மருந்தின் மூலம் தடுப்பது பற்றிய ஆய்வு

நோயாளி அடையாளப்படுத்துதல் :

பங்கேற்பாளர் எண் :

ஒரு ஆராய்ச்சி ஆய்வில் பங்கேற்கும்படி நீங்கள் கேட்டுக்கொள்ளப்படுகிறீர்கள்.பங்கு பெற உங்களுக்கு விருப்பமா ,என்பதை முடிவு செய்ய உங்களுக்கு உதவ இந்தப்படிவம் தகவல்களைக் கொண்டுள்ளது.நேரம் எடுத்துக்கொண்டு ,இந்த படிவத்தை கவனமாக படித்து உங்களுக்கு இருக்கும் ஐயப்பாடுகளை ஆய்வு மருத்துவரையோ அல்லது ஏதுனும் ஊழியர்களையோ கேட்கவும்.

இந்த ஆய்வை பற்றி :-

இந்த ஆய்வில் பங்கேற்க நீங்கள் அனுமதிக்கப்படுவதற்கு இந்த காரணங்கள் இருக்கக்கூடும் .

- தாங்கள் மார்பகப் புற்றுநோய்க்காக டாக்சோரூபிசின் சிகிச்சை எடுத்துக்கொள்வதால் ஏற்படபோகும் இதய பாதிப்பை முன்கூட்டியே அறிந்து கொள்ளவும் அதனைத் தடுப்பதற்கான வழிமுறைகளை பின்பற்றுவதர்க்காகவும்.

நான் என்ன செய்யுமாறு கேட்டுகொள்ளப்படுவேன் .

- மூன்று வாரத்திற்கு ஒரு முறை வந்து மாத்திரைகளை வாங்கிக்கொள்வது .
- நேரத்திற்கு சரியாக துங்குவது.
- மருத்துவர் அறிவுரை இல்லாமல் எந்த ஒரு புதிய மருந்தோ மாத்திரையோ உட்கொள்ளாமல் இருப்பது .
- நீங்கள் ஒவ்வொரு முறை வரும்பொழுதும் நீங்கள் எடுத்தமருந்தின் காலிஅட்டைகளை கொண்டுவருவது .
- வருகையின் இடையில் உங்களுக்கு ஏதேனும் பக்கவிளைவுகள் இருக்கிறதா என்பதை அறிந்து கொள்வதற்காக, ஆய்வு ஊழியர்களிடமிருந்து வரும் தொலைபேசி அழைப்புகளை பெற்றுகொள்வது.

பங்கேற்பதற்கான தகுதிகள் :-

- புற்றுநோய் மருத்துவப்பிரிவில்

  உள்நோயாளியாக அனுமதிக்கப்பட்டு டாக்சோரூபிசின்

  சிகிச்சைபெறும் மார்பக புற்றுநோயளிகள் மட்டும்.

கீழ்க்கண்டவற்றுள் ஏதேனும் குறைபாடு உங்களிடம் இருந்தால்

நீங்கள் இந்த ஆய்வில் இருந்து விலக்கப்படுவீர்கள்:-

- உங்கள் வயது 20க்கு கீழிருந்தால் மற்றும் 65 க்கு மேலிருந்தால்
- கர்ப்பிணி மற்றும் பாலூட்டும் தாய்மார்கள்
- கல்லீரல் குறைபாடு உடையவர்கள்
- சிறுநீரகக் கோளாறு உடையவர்கள்
- மருந்தினால் ஏற்படும் ஒவ்வாமை.
- இருபுறமும் சிறுநீரகத் தமனிச் சுருக்கம் உடையவர்கள்
- உயர் இரத்த அழுத்தம் மற்றும் இதய நோயாளிகள்
- இரத்தத்தில் அதிகமான பொட்டசியம் இருப்பது

நீங்கள் உங்கள் ஆய்வு வருகைகளுக்கு வரும்போது ஆய்வு மருத்துவரால் அல்லது ஆய்வு ஊழியர்களால் பின்வருபவைகளில் ஏதுனும் ஒன்றோ இல்லை முழுவதுமாகவோ உட்படுத்தபடுவீர்கள்.

- உங்கள் மருத்துவ வரலாற்றை மறுஆய்வு செய்வது.
- ஆய்வு மருந்துகளையும் அறிவுரைகளையும் வழங்குவது.
- உங்களது இதயத்துடிப்பு ,இரத்தஅழுத்தம் ,உடல்எடை,உயரம் ஆகியவற்றை அளப்பது .
- இரத்தம் மற்றும் சிறுநீர் மாதிரிகள் பரிசோதனை.
- எலெக்ட்ரோ கார்டியோக்ராம் (இருதய சுருள் படம்), மற்றும் எக்கோகார்டியோக்ராம் (இருதய ஸ்கேன்).

மருந்துகள் எப்பொழுதாவது கீழ்க்கண்ட பின்விளைவுகளையோ அல்லது பக்கவிளைவுகளையோ உண்டாக்கலாம்.

- வாந்தி,மயக்கம்.
- நெஞ்சுபடபடப்பு , தலை சுற்றல் .
- இரத்தத்தில் அதிகமான பொட்டசியம் இருப்பது.
- தோலின் ஒவ்வாமை.
- எலும்புமஜ்ஜை பாதிப்பால் இரத்தஅணுக்கள் குறைவது
- வாய்ப்புண்,
- முடி உதிர்வு

- வறட்டு இருமல்
- முகம் மற்றும் உதடு வீக்கம்
- இரத்த அழுத்தம் குறைதல்
- சுவையில் மாற்றம்

ஆய்வின் போது பின்விளைவுகள் ஏற்ப்பட்டால் ஆய்வு மருந்து உட்கொள்வதை நிறுத்திவிட்டு உடனடியாக மருத்துவரை அணுகவேண்டும்.உங்கள் மருத்துவர் வேறு மருந்து கொடுத்து பின்விளைவுகளை நிறுத்த முயற்சிப்பார்.

இரத்தப் பரிசோதனை செய்வதால் உண்டாகும் எதிர்விளைவுகள்

பெரும்பாலானவர்களுக்கு இரத்தம் எடுப்பதற்காக ஊசி குத்துவது எவ்வித மோசமான பிரச்சனைகளையும் ஏற்படுத்துவதில்லை.ஆயினும் , சில நேரங்களில் இரத்தம் எடுக்கப்பட்ட இடத்தில் , இரத்தக் கசிவு , இரத்தம் கன்றிப் போதல் ,அசௌகரியம் , நோய்தொற்றுக்கள் மற்றும் /அல்லது வலி ஆகியவை உண்டாகலாம். நீங்கள் தலைசுற்றுவதாகவும் உணரலாம்

எலட்ரோகார்டியோக்ராம் / ஈ . சி . ஜி

எலட்ரோகார்டியோக்ராம் / ஈசிஜி பரிசோதனை இதயத்துடிப்பு அல்லது இதய தாளத்தின் மின்சார தடமறிதல்.அவை ஈகேஜி பரிசோதனை எனவும் அழைக்கபடுகின்றன. ஈசிஜி பரிசோதனை செய்து கொள்வதற்கு , உங்கள் உடலின் பல்வேறு பகுதிகளில் ஒட்டுக்கள் வைக்கப்படும்.

எக்கோ கார்டியோக்ராம் .

எக்கோ பரிசோதனை செய்வதற்கு உங்கள் உடலில் நெஞ்சுப் பகுதியில் டிரான்ஸ்டூசர் எனும் சிறிய கருவி கொண்டு ஆய்வு செய்யப்படும் .எக்கோ கருவியிலிருந்து எவ்விதமானஅபாயமான அயனியாக்கும்கதிர்வீச்சும் உண்டாவதில்லை.எக்கோ கருவியானது மீயொலி எழுப்பி அதனை உடலில் செலுத்திபின் அதன் எதிரொலிப்பை கணக்கிட்டு இருதய தசையின் தன்மையை மற்றும் இதய தசை நிறை குரியீட்டுயெண் ஆகியவற்றை அளவிட உதவும்.

உலகளாவிய தரவுகள் பாதுகாப்பு அறிக்கை

இந்த ஆய்வை நடத்துவதன் ஒரு பகுதியாக, உங்கள் மருத்துவத் தகவல்களை ஆய்வு மருத்துவர் தவிர பிறருடன் பகிர்ந்து கொள்வது

அவசியமாகிறது. உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்கள் எவ்வாறு பயன்படுத்தப்படும் மற்றும் இந்த ஆராய்ச்சி ஆய்வுக்காக அவை யாருக்குத் தரப்படும் (வெளிப்படுத்துதல்) என்பவை பற்றி, தரவுகள் பாதுகாப்பு அறிக்கை விளக்கமளிக்கிறது. உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்களைப் பார்வையிட உங்கள் உரிமைகள் உட்பட உங்களது பாதுகாப்பு உரிமைகளையும் இது விவரிக்கிறது.

நீங்கள் யார் என்று கண்டறியப் பயன்படுத்தபடும் உங்களைப் பற்றிய தகவல்களே உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்களாகும். இந்த ஆய்விற்குத் தேவைப்படும் தற்போதைய உங்கள் மருத்துவப் பதிவேடுகளில் உள்ள தகவல்கள் இந்த ஆய்வின்போது உருவாக்கப்படும் அல்லது சேகரிக்கப்படும் புதிய தகவல்கள் ஆகியவற்றை உள்ளடக்கியவையே இந்த ஆய்விற்குத் தேவையான தகவல்களாகும்.

இந்த ஆய்விற்கான ஒப்புதல் படிவத்தில் கையொப்பமிடுவதன் வாயிலாக , இந்த தரவுகள் பாதுகாப்பு அறிக்கையில் விவரிக்கப்பட்டுள்ள உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்களை பயன்பாட்டுக்கும் வெளிப்படுத்துதலுக்கும் நீங்கள் அனுமதி (“அங்கீகாரம் “) அளிக்கிறீர்கள் . இப்பயன்பாடுகளை நீங்கள் அனுமதிக்க விரும்பவில்லை என்றால், இந்த ஆய்வில் நீங்கள் பங்கேற்கக் கூடாது.

இந்த ஆய்வில் பங்கேற்க நீங்கள் ஒப்புக் கொண்டால், உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்கள் கீழ்க்கண்ட வழிகளில் பயன்படுத்தப்படும் மற்றும் வெளிப்படுத்தப்படும்.

- ஆய்வின் போது, ஆய்வை நடத்துவதற்காக, உங்கள் மருத்துவப் பதிவேடுகளையும், உருவாக்கப்பட்ட அல்லது சேகரிக்கப்பட்ட தகவல்களையும் ஆய்வு மருத்துவர் மற்றும் ஊழியர்கள் பயன்படுத்துவார்கள்.

- ஒப்புதல் படிவத்தில் விவரிக்கப்பட்ட ஆய்வின் அறிவியல் நோக்கங்களுக்கு ஆதரவளிக்கும் ஆராய்ச்சிக் காரணங்களுக்காகவும் ,ஆய்வில் சேர்க்கப்பட்ட மருந்து அல்லது சிகிச்சையின் பாதுகாப்பு மற்றும் பலன்களை மதிப்பீடு செய்யவும், ஆய்வில் சேர்க்கப்பட்டுள்ள நோய்(கள்) பற்றி சிறப்பாக புரிந்து கொள்ளவும் அல்லது எதிர்கால ஆய்வுகளை வடிவமைத்து மேம்படுத்தவும் இந்த ஆய்வுத் தரவுகள் பயன்படும்.

- உங்களை அடையாளம் காணாத ஆய்வுத் தரவுகள் மருத்துவ இதழ்களில் வெளியிடப்படலாம் அல்லது அறிவியல் விவாதங்களின் ஒரு பகுதியாக மற்றவர்களுடன் பகிர்ந்து கொள்ளப்படலாம்.

- உங்கள் மருத்துவ பதிவேடுகள் மற்றும் ஆய்வுத் தரவுகள் கணினிகளில் வைக்கப்பட்டு செயல்முறைப்படுத்தப்படலாம்.

ஆய்வு தொடர்பான உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்கள், ஆய்வு மருத்துவரிடம் இருக்கும்வரை, அவற்றை நீங்கள் பார்வையிடவும் அவற்றின் நகலைப் பெறவும், உங்களுக்கு உரிமையுண்டு. இருப்பினும் ,ஆய்வின் அறிவியல் ஒருமைப்பாட்டை உறுதிசெய்யும் பொருட்டு, ஆய்வு முடிவடையும் வரை சில ஆய்வுத் தகவல்களை நீங்கள் மறுஆய்வு செய்ய முடியாது.

ஆய்வு மருத்துவருக்கு எழுத்து மூலம் அறிவிப்பை அளித்து உங்கள் அங்கீகாரத்தை எப்போது வேண்டுமானாலும் நீக்கி விடலாம். நீங்கள் உங்கள் அங்கீகாரத்தை நீக்கிக் கொண்டால், ஆய்வின் அறிவியல் ஒருமைப்பாட்டை பாதுகாப்பதற்காக, ஆய்வு மருத்துவர் அல்லது ஊழியர்கள் இந்த ஆய்வுடன் தொடர்புடைய உங்கள் தனிப்பட்ட மருத்துவத் தகவல்கள் சிலவற்றை பயன்படுத்தும் அல்லது உரிமை வழங்கல் தேவை இல்லாதவரை , உங்கள் ஆய்வு மருத்துவர் அல்லது ஊழியர்கள் உங்கள் தனிப்பட்ட ஆரோக்கியத் தகவல்களைப் பயன்படுத்தவோ அல்லது வெளிப்படுத்தவோ மாட்டார்கள்.

இணைப்பு - 2

நோயாளி தகவல் மற்றும் ஒப்புதல் படிவம்

ஆய்விடத் தகவல் மற்றும் தொடர்பு விவரங்கள்

மார்பக புற்றுநோயாளிகளுக்கு டாக்சோரூபிசின் சிகிச்சையினால் இருதயத்தில் ஏற்படும் பாதிப்புகளை எனலாபிரில் மருந்தின் மூலம் தடுப்பது பற்றிய ஆய்வு.

ஆய்வு குறித்த ஐயப்பாடுகள் மற்றும் விவரங்களைப் பற்றி

கேட்பதற்கான ஆய்வு மருத்துவர்களின் தொடர்பு விவரங்கள்.

ஆய்வு மருத்துவரின் பெயர் :-

_____

முகவரி :-

_____

_____

தொடர்பு எண் :-

_____

ஓர் பங்கேற்பாளராக உங்கள் உரிமைகளைப் பற்றி கேட்பதற்கான

ஈஆர்பி தொடர்பு விவரங்கள் :-

ஐஆர்பி தொடர்பு நபரின் பெயர் :-

_____

_____

தொடர்பு எண் :-

_____

இணைப்பு -3.

நோயாளி தகவல் மற்றும் ஒப்புதல் படிவம்

மார்பக புற்றுநோயாளிகளுக்கு டாக்சோரூபிசின் சிகிச்சையினால் இருதயத்தில் ஏற்படும் பாதிப்புகளை எனலாபிரில் மருந்தின் மூலம் தடுப்பது பற்றிய ஆய்வு

இந்தப் பக்கத்தில் கையொப்பமிடுவதன் மூலமாக ,பின்வருவனவற்றை நான் உறுதி செய்கிறேன்.

- மேற்படி ஆய்விற்கான _____ தேதியிடப்பட்ட இந்த நோயாளி தகவல் மற்றும் ஒப்புதல் படிவத்திலுள்ள அனைத்துத் தகவல்களையும் நான் படித்துப் புரிந்து கொண்டிருக்கிறேன் எனவும்

அதைப்பற்றி சிந்திக்க எனக்கு கால அவகாசம் இருந்தது எனவும் நான் உறுதியளிக்கிறேன்.

- கேள்விகள் கேட்பதற்கான வாய்ப்பு எனக்கு இருந்தது மேலும் எனது கேள்விகளனைத்தும் எனது திருப்திக்குத் தக்கவாறு பதிலளிக்கப்பட்டிருக்கின்றன.

- இந்த ஆய்வில் என் பங்கேற்பு தன்னார்வம் சார்ந்தது எனவும் பங்கேற்பை எப்போது வேண்டுமானாலும் எவ்விதக் காரணமும் அளிக்காமல், என் மருத்துவக் கவனிப்பு அல்லது சட்ட உரிமைகள் பாதிக்கப்படாமல் விலகிக் கொள்ள நான் சுதந்திரமானவர் என்பதை நான் புரிந்து கொள்கிறேன்.

- வேண்டிக்கொள்ளப்பட்டபடி, ஆய்வு நடைமுறைகளை பின்பற்றவும் ,மற்றும் மருத்துவர் ,செவிலிகள் ,அல்லது மற்ற ஊழிய உறுப்பினர்களுக்கு தேவையான தகவல்களை வழங்கவும் நான் தன்னார்வத்துடன் ஒப்புக் கொள்கிறேன்.

- இந்த ஆய்விலிருந்து எழும் எந்தவொரு தரவு அல்லது முடிவுகளின் உபயோகத்தினையும்,இது போன்ற உபயோகமான தரவு பாதுகாப்பு அறிக்கையில் குறிப்பிட்டபடி மட்டுமாக உபயோகிக்கப்படும்

பட்சத்தில் அவைகளை நான் தடுக்காமலிருக்க நான் ஒப்புக் கொள்கிறேன்.

- மேற்கண்ட ஆய்வில் பங்கெடுக்க நான் ஒப்புக் கொள்கிறேன்.
- எனக்காக வைத்துக்கொள்வதற்காக இந்த நோயாளி தகவல் மற்றும் ஒப்புதல் படிவத்தின் ஓர் நகலை நான் பெற்றுக் கொள்கிறேன்.

தேதி:-

நோயாளி / சட்டபூர்வமாக ஏற்றுக் (அல்லது) நோயாளி கற்காதவரானால்

கொள்ளக்கூடிய பிரதிநிதியின்(எல்ஏஆர்) (நோயாளி/எல்ஏஆர்)தாமாகவே

கையொப்பம் பாகுபாடற்ற சாட்சியின் முன்னிலையில் தேதியிட வேண்டும்)

நோயாளி வாய்மூலமான ஒப்புதல்

கொடுத்திருக்கிறார் என்பதை குறிப்பதற்காக

பெருவிரல் ரேகை)

நோயாளி பெயர்:-

நோயாளி எண் மற்றும் பெயர் முதலெழுத்துக்கள்

பிறந்த தேதி & வயது:-

எல்ஏஆர்-இன் பெயர் ,உரித்தானால்

(அச்சில் அல்லது தட்டச்சில்)

சட்டப்பூர்வமாக ஏற்றுக்கொள்ளக்கூடிய

பிரதிநிதி கையொப்பமிட்டால்,

நோயாளியுடனான உறவுமுறை.

தகவலளிக்கப்பட்ட ஒப்புதல் விவரத்தை

நடத்தும் ஆராய்ச்சியாளர் / அவரால்

நியமிக்கப்பட்டவரின் பெயர்(அச்சில் அல்லது

தட்டச்சில்).

MASTERT CHARTS

CONTROL GROUP (WITHOUT ENALAPRIL)																			
S.No	**IP No**	**Age**	**Sex**	**Height**	**Weight**	**BSA**	**Total Doxo Dose**	**TnI Baseline**	**TnI 24 Hrs**	**TnI End**	**LVEF Baseline**	**LVEF 3rd Cycle**	**LVEF 6th Cycle**	**LVEF 6th Month**	**LVEF 9th Month**	**FS Baseline**	**FS 3rd Cycle**	**FS 6th Cycle**	**FS 6th Month**	**FS 9th Month**
1	71892	45	F	166	68	1.8	540	0.2	1.5	1.7	64	60	58	54	48	35	32	30	27	25
2	71907	48	F	158	54	1.5	450	0.4	0.5	0.5	63	61	59	60	60	34	32	31	31	32
3	71893	43	F	157	55	1.5	450	0.1	0.1	0.1	61	60	57	58	57	33	32	30	30	30
4	71871	38	F	163	58	1.6	480	0.2	0.2	0.3	58	58	56	55	54	30	30	29	28	28
5	71870	64	F	154	60	1.6	480	0.3	0.5	0.5	62	60	59	57	57	34	33	32	31	31
6	71913	58	F	164	70	1.8	540	0.4	1.7	2.1	61	57	54	52	47	34	31	29	28	25
7	71868	59	F	165	52	1.5	450	0.1	0.2	0.2	60	59	58	57	57	33	32	31	30	30
8	82772	55	F	156	60	1.6	480	0.2	0.2	0.3	62	61	59	57	58	34	33	31	30	31
9	82998	49	F	166	53	1.5	450	0.2	0.3	0.4	64	62	60	60	58	36	34	32	32	31
10	83015	35	F	155	62	1.6	480	0.4	0.3	0.3	69	67	65	64	64	40	38	35	35	36
11	82997	64	F	161	62	1.7	510	0.5	1.7	2.2	62	59	55	53	48	35	33	30	28	25
12	83006	39	F	154	54	1.6	480	0.3	0.4	0.4	60	58	56	55	57	33	32	31	30	31
13	83007	65	F	151	52	1.5	450	0.4	0.4	0.3	61	59	58	57	57	33	32	31	30	30
14	83034	57	F	152	50	1.5	450	0.2	0.3	0.2	60	58	56	57	57	33	31	31	31	30

15	83000	60	F	166	56	1.6	480	0.4	0.3	0.3	59	56	55	55	55	32	30	29	29	28
16	83026	49	F	158	50	1.5	450	0.3	0.3	0.4	61	58	58	57	57	33	32	32	30	30
17	73708	54	F	155	57	1.6	480	0.2	0.2	0.3	63	60	60	59	60	35	33	34	32	33
18	66840	65	F	156	68	1.7	510	0.4	1.3	1.7	58	53	50	48	45	31	28	27	25	24
19	68724	63	F	168	74	1.9	570	0.5	1.4	1.8	58	55	52	49	46	31	29	27	26	24
20	66820	34	F	148	53	1.5	450	0.1	0.2	0.2	70	68	66	66	66	40	38	37	36	36
21	66839	50	F	150	50	1.4	420	0.1	0.1	0.2	58	57	54	54	54	32	32	31	30	29
22	66724	52	F	165	70	1.8	540	0.4	1.4	1.9	61	55	52	49	47	33	30	28	27	24
23	82982	64	F	159	57	1.6	480	0.1	0.1	0.2	63	61	60	59	60	35	33	32	31	32
24	82986	60	F	166	66	1.7	510	0.4	1.4	1.7	59	55	53	52	46	32	30	29	27	25
25	82983	54	F	158	52	1.5	450	0.2	0.2	0.2	66	63	61	62	62	36	35	33	33	33
26	82990	58	F	160	56	1.6	480	0.1	0.1	0.2	58	54	52	54	52	31	30	29	29	28
27	82957	55	F	153	54	1.5	450	0.2	0.2	0.3	65	63	60	61	60	35	33	32	31	31
28	73718	61	F	162	70	1.8	540	0.5	1.5	1.9	60	57	55	51	48	33	30	29	26	25
29	66831	37	F	158	60	1.6	480	0.3	0.3	0.4	62	57	54	53	52	33	30	29	28	27
30	66729	65	F	160	70	1.8	540	0.5	1.6	1.9	61	55	53	53	48	33	29	28	27	25

ENALAPRIL GROUP																				
S.No	**IP No**	**Age**	**Sex**	**Height**	**Weight**	**BSA**	**Total Doxo Dose**	**TnI Baseline**	**TnI 24 Hrs**	**TnI End**	**LVEF Baseline**	**LVEF 3rd Cycle**	**LVEF 6th Cycle**	**LVEF 6th Month**	**LVEF 9th Month**	**FS Baseline**	**FS 3rd Cycle**	**FS 6th Cycle**	**FS 6th Month**	**FS 9th Month**
1	63806	64	F	160	62	1.7	510	0.2	1.4	1.3	63	57	55	61	64	34	30	29	32	34
2	64852	62	F	155	58	1.6	480	0.1	0.2	0.2	65	63	63	66	66	38	36	35	37	38
3	64775	45	F	150	55	1.5	450	0.1	0.2	0.2	67	65	65	66	68	38	36	37	38	39
4	64795	60	F	156	60	1.6	480	0.3	1.4	1.2	64	60	57	65	65	35	33	30	34	35
5	64771	63	F	158	65	1.7	510	0.1	1.5	1.7	60	55	53	61	62	34	29	28	32	34
6	64796	62	F	162	70	1.8	540	0.3	1.8	1.5	58	52	48	60	61	33	31	25	31	32
7	64740	55	F	165	56	1.6	480	0.2	0.2	0.2	59	60	58	61	62	30	30	31	32	33
8	64738	45	F	159	62	1.6	480	0.1	0.1	0.1	65	63	62	65	66	36	34	33	34	35
9	64734	58	F	164	70	1.8	540	0.4	0.4	0.4	58	56	54	59	61	33	32	31	32	34
10	64752	35	F	155	60	1.6	480	0.5	0.5	0.5	60	58	58	60	62	33	32	32	33	33
11	70842	42	F	158	62	1.6	480	0.5	0.5	0.5	62	59	58	60	63	36	35	34	33	34
12	70843	50	F	156	58	1.6	480	0.2	0.3	0.3	59	57	57	58	60	33	32	31	32	33
13	70870	36	F	154	52	1.5	450	0.3	0.3	0.3	61	60	58	60	60	33	32	32	32	33

14	70846	40	F	152	64	1.6	480	0.3	0.3	0.3	66	63	62	63	65	36	34	34	38	38
15	78190	61	F	165	74	1.9	570	0.2	2.2	1.7	58	53	47	56	60	31	29	25	29	31
16	74340	57	F	160	58	1.6	480	0.3	0.3	0.3	65	62	61	62	62	38	37	37	39	38
17	79146	48	F	154	50	1.5	450	0.1	0.1	0.1	60	60	56	58	58	33	32	30	31	31
18	79142	58	F	158	54	1.5	450	0.1	0.1	0.1	57	56	54	56	60	30	30	29	31	31
19	79129	52	F	156	60	1.6	480	0.2	0.5	0.5	64	61	55	60	61	37	35	30	35	37
20	79127	64	F	162	64	1.7	510	0.3	1.9	1.9	59	54	50	58	59	32	31	26	31	32
21	75176	55	F	158	60	1.6	480	0.1	0.2	0.2	62	60	58	60	61	33	33	31	33	33
22	79125	53	F	152	55	1.5	450	0.2	0.2	0.2	58	55	53	55	58	31	30	28	31	31
23	79143	60	F	162	58	1.6	480	0.4	0.7	0.7	63	61	57	60	62	37	33	32	34	35
24	79168	65	F	160	63	1.7	510	0.2	1.4	1.4	64	58	54	60	62	36	33	30	33	35
25	79138	38	F	156	58	1.6	480	0.2	0.2	0.2	58	58	56	56	60	32	32	30	31	33
26	79128	36	F	156	54	1.5	450	0.4	0.4	0.4	60	58	56	59	61	33	31	30	31	33
27	79136	57	F	154	62	1.6	480	0.4	0.7	0.9	63	61	59	61	62	34	32	32	33	34
28	79169	61	F	160	66	1.7	510	0.5	1.1	1.1	62	58	56	59	62	33	30	29	28	33
29	78170	35	F	158	58	1.6	480	0.3	0.3	0.3	60	59	58	58	61	33	31	31	32	34
30	78185	47	F	154	60	1.6	480	0.1	0.7	0.7	64	62	61	63	63	36	32	31	34	36

ABBREVIATIONS

ACE	-Angiotensin converting enzyme
ACEI	-Angiotensin converting enzyme inhibitor
AIP	-Aldosterone induced protein
ALL	-Acute lymphoblastic leukemia
AML	-Acute myeloblastic leukemia
ARB	-Angiotensin receptor blocker
AT1 R	-Angiotensin 1 receptor
ATP	-Adenosine tri phosphate
CAD	-Coronary artery disease
CHF	-Congestive heart failure
CLL	-Chronic lymphoblastic leukemia
CT	-Computed tomography
DNA	-Deoxyribonucleic acid
DOX	-Doxorubicin
ECG	-Electrocardiogram
ECHO	-Echocardiogram
EDV	-End diastolic volume
ELISA	- Enzyme-linked immunosorbent assay
ESV	-End systolic volume
FDA	-Food and drug administration
FGF	-Fibroblast growth factor
FNAC	-Fine needle aspiration cytology

FS	-Fractional shortening
HSP	-Heat shock protein
IRP	-Iron regulatory proteins
IUGR	-Intra uterine growth retardation
LVEF	-Left ventricular ejection fraction
LVIDd	-Left ventricular internal diameter in diastole
LVIDs	-Left ventricular internal diameter in systole
MAPK	-Mitogen activated protein kinase
MRI	-Magnetic resonance imaging
NADPH	-Nicotinamide adenine dinucleotide phosphate
NOS	-Nitric oxide synthase
NSAID	-Non steroidal antiinflammatory drugs
PDGF	-Platelet derived growth factor
RAS	-Renin angiotensin system
RNA	-Ribonucleic acid
ROS	-Reactive oxygen species
sNAP	-s-Nitrosyl n- acetyl penicillamine
SOD	-Super oxide dismutase
TGF	-Transforming growth factor
TnI	-Troponin I
VT	-Ventricular tachycardia
WHO	-World health organization
5 FU	-5 Fluorouracil

ETHICAL CLEARANCE LETTER

Ref. No. 4105/E4/3/2013 **Govt. Rajaji Hospital, Madurai.20. Dated: . 04.2013**

Institutional Review Board / Independent Ethics Committee.
Dr. N. Mohan, M.S., F.I.C.S., F.A.I.S.,
Dean,
Madurai Medical College &
Govt Rajaji Hospital, Madurai 625020.
Convenor

Sub: Establishment-Govt. Rajaji Hospital, Madurai-20-
Ethics committee-Meeting Minutes- for March 2013
Approved list -regarding.

The Ethics Committee meeting of the Govt. Rajaji Hospital, Madurai was held on 27.03.2013 at 10.00 am to 12.00.pm at the Surgery Seminar Hall, Govt. Rajaji Hospital, Madurai. The following members of the committee have attended the meeting.

1.Dr. V. Nagarajan, M.D., D.M (Neuro) Ph: 0452-2629629 Cell.No 9843052029	Professor of Neurology (Retired) D.No.72, Vakkil New Street, Simmakkal, Madurai -1	Chairman
2. Dr.Mohan Prasad , M.S M.Ch Cell.No.9843050822 (Oncology)	Professor & H.O.D of Surgical Oncology(Retired) D.No.72, West Avani Moola Street, Madurai -1	Member Secretary
3. Dr.L. Santhana Lakshmi,MD Cell.No 9842593412	Associate Professor of Physiology/V.P Madurai Medical College	Member
4. Dr. Parameswari M.D (Pharmacology) Cell.No.9994026056	Director of Pharmacology Madurai Medical College	Member
5. Dr.Moses K.Daniel MD(Gen.Medicine) Cell.No 09842156066	Professor & H.O.D of Medicine Madurai Medical College	Member
6. Dr.D. Soundara Rajan,MS(Gen.Surgery) Cell.No 9842120127	Professor & H.O.D of Surgery Madurai Medical College	Member
7. Dr.Angayarkanni MD(O&G) Cell.No 9443567724	Professor & H.O.D of O&G Madurai Medical College	Member
8. Dr.P.V. Pugalenthi M.S, (Ortho) Cell.No 9443725840	Professor & H.O.D Ortho Madurai Medical College	Member
9. Dr. M. Sundarajan M.S., Mch Cell.No 9994924369 (Neuro Surgery)	Professor (Neuro Surgery) Madurai Medical College	Member
10 Thiru..Pala. .Ramasamy , BA.,B.L., Cell.No 9842165127	Advocate, D.No.72.Palam Station Road, Sellur, Madurai -2	Member
11. Thiru. P.K.M. Chelliah ,B.A Cell.No 9894349599	Businessman, 21 Jawahar Street, Gandhi Nagar, Madurai-20.	Member

The following Project was approved by the committee

Name of P.G.	Course	Name of the Project	Remarks
Dr. J. Arun Kumar	PG in MD Pharmacology Medical College, & Govt. Rajaji Hospital, Madurai.	Study on Cardio protective effect of Enalapril in patients with breast cancer on doxorubicin chemotherapy.	Approved

Please note that the investigator should adhere the following: She/He should get a detailed informed consent from the patients/participants and maintain Confidentially.

1. She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the institution to Government.
2. She/He should inform the institution Ethical Committee in case of any change of study procedure site and investigation or guide.
3. She/He should not deviate for the area of the work for which applied for Ethical clearance.

She/He should inform the IEC immediately, in case of any adverse events pr Serious adverse reactions.

4. She/he should abide to the rules and regulations of the institution.
5. She/He should complete the work within the specific period and apply for if any Extension of time is required She should apply for permission again and do the work.
6. She/He should submit the summary of the work to the Ethical Committee on Completion of the work.
7. She/He should not claim any funds from the institution while doing the word or on completion.
8. She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

Member Secretary **Chairman**

DEAN/Convenor
Govt. Rajaji Hospital,
Madurai- 20.

10/4/13

To
The above Applicant
-thro. Head of the Department concerned.

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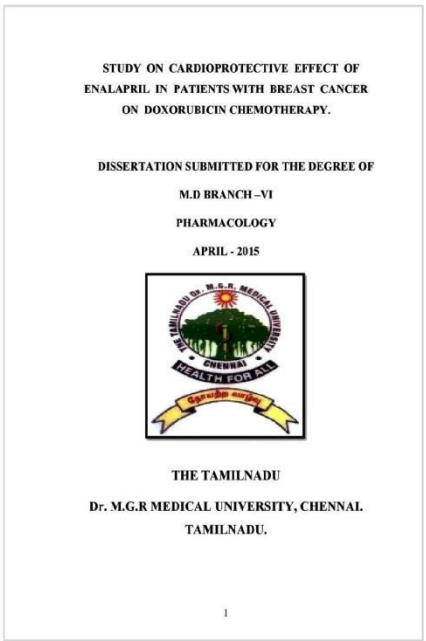

STUDY ON CARDIOPROTECTIVE EFFECT OF ENALAPRIL IN PATIENTS WITH BREAST CANCER ON DOXORUBICIN CHEMOTHERAPY.

DISSERTATION SUBMITTED FOR THE DEGREE OF

M.D BRANCH – VI

PHARMACOLOGY

APRIL - 2015

THE TAMILNADU

Dr. M.G.R MEDICAL UNIVERSITY, CHENNAI.

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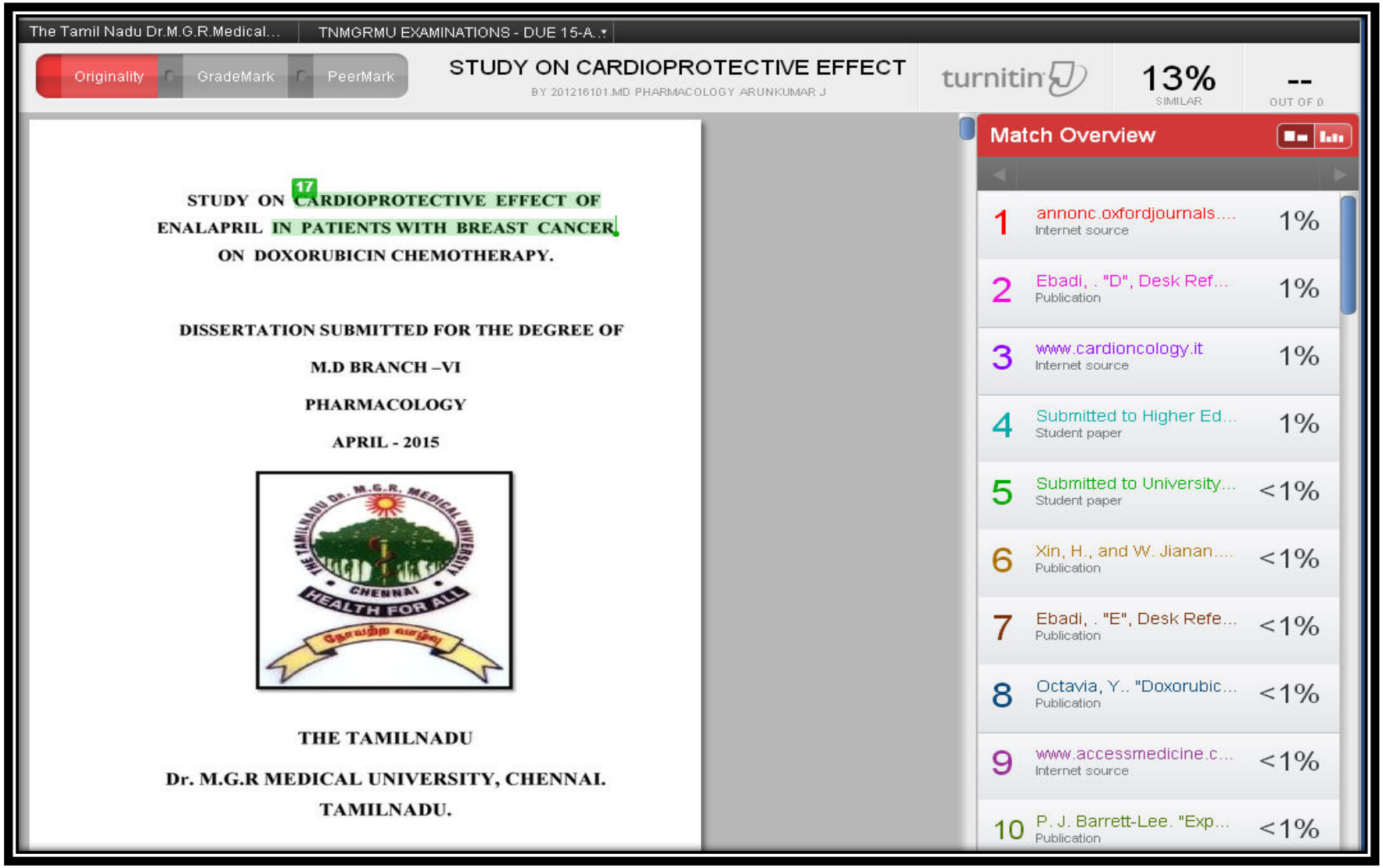

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